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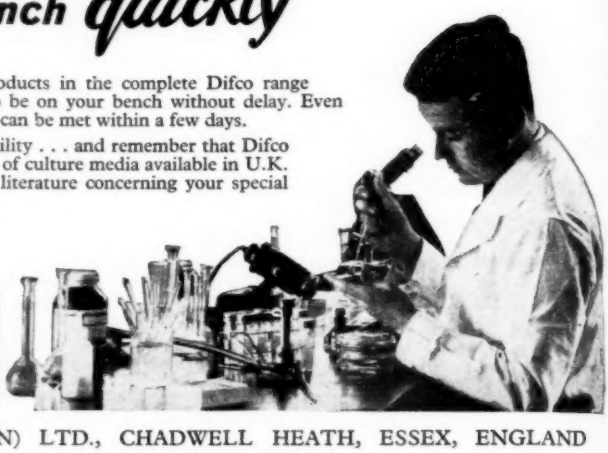

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CREATIVE ABILITY

IN his presidential address to Section F (Economics) at the British Association meeting at York in 1959, Prof. John Jewkes asked some searching questions about accepted correlations between industrial output and the number of scientists and technologists, and suggested that we needed better criteria for determining how much of its resources the community should devote to science and technology. Prof. Jewkes's views on the shortage of scientists were promptly challenged by Sir Solly Zuckerman, particularly in the light of figures afterwards issued by the Scientific Man-power Committee of the Advisory Council on Scientific Policy, although Sir Solly agreed entirely as to the need for more satisfactory criteria. In the past two years there has been no serious suggestion that the supply of scientists or technologists is increasing unduly. Shortages of particular categories of scientists, as, for example, teachers of mathematics, have, however, emphasized the soundness of some of Prof. Jewkes's remarks about the decisions required in striking the right balance between the numbers of scientists and technologists.

This is equally true of Prof. Jewkes's comment on the extreme importance of employing the scientists and technologists we already have to the best effect, a matter on which Prof. Jewkes already had some doubts. This year, at Norwich, his successor as president of Section F devoted the whole of his presidential address to an examination of the conditions which affect the economic use of intellect. Prof. C. F. Carter also has reservations as to the extent of the alleged shortage of scientists and technologists. He finds no evidence that the present educational system of Britain is biased towards arts subjects. On the contrary, he believes that the specialized study of science and technology is in danger of attracting an undue proportion of able minds, and that, at the higher levels of ability, it is more significant to examine the general shortage rather than the specialist deficiencies.

Prof. Carter begins, indeed, by stressing the importance of the supply of first-rate ability. All too often in discussions on trained man-power, or university or technical education, or in such matters as training for management, there is a disposition to ignore the importance of really first-rate ability and its implications to-day and to concentrate on the needs and the supply of the middle and lower ranges of ability. Prof. Carter rightly points out that, leaving aside for the moment the question of how limited is the supply of such ability, its importance relative to other natural resources increases as technology becomes more complex; and because ideas and methods of organization and working are capable of being copied, those of the highest ability, ingenuity and inventiveness, who are an example to others, are of special importance. It is the supply of first-rate ability which determines the speed of change, all other shortages being of secondary importance.

Nevertheless, Prof. Carter contends that we have given surprisingly little thought to the nature and proper use of this factor, and that there is considerable need for further study and research. It is, of course, difficult to define what is meant when in common speech we talk of an 'able man'. We do not mean one whose only skill is the perfect performance of a very narrow task. The shortage of the higher forms of ability, Prof. Carter points out, is a shortage of men capable of dealing with new and unexpected tasks within a certain range, and who are not confined within too narrow a specialization. We are looking for an intelligence which is capable of analysing a new situation and reacting to it in a sensible way, and this quite clearly is something which will be found independently of the particular disciplines in which that intelligence has been trained.

We are thus at once reminded of the importance of general education itself—but Prof. Carter is not deflected from his main discussion. Something more is required than developed intelligence, and he notes that since people can be intelligent but ineffective, the causes of ineffectiveness themselves may well be worthy of more attention. In fact, serious errors are frequently made in both directions in our judgment as to how intelligent men will react to the stress of responsibility; while Prof. Carter does not doubt that scientific selection procedure can reduce these errors, he also considers that personnel selection at the highest levels has far to go before it can claim to be very scientific.

Properly speaking, the waste of ability due to failure to find appropriate occupation—because of adverse social or economic conditions during the educational period—is scarcely a matter of the economic use of brains, though it is the aspect that has most commonly been considered. Prof. Carter, moreover, is hesitant in accepting the conclusion, drawn from reports such as *Early Leaving*, which demonstrate a continuing connexion between a long and successful educational career and high social class of the parents, that the supply of able people will necessarily be increased by a wider and better educational system. He questions, in fact, whether improved educational provision will necessarily produce this result, though he agrees that we might be able to alter the supply of ability if we could alter the social structure or environment in some relevant way. As yet, however, we do not know which of the inter-related characteristics of a poor social environment are most important in restricting the development of a child, and it is highly probable that while

change of this kind may, in fact, be proceeding, it is a long-term process. Further, it is equally possible that the ultimate result of all such changes will be increased numbers in the middle ranges of ability rather than in the high levels of ability with which Prof. Carter is primarily concerned in this address.

For such reasons, Prof. Carter is indisposed to accept, on the evidence available, that there is plenty of high ability available, at present running to waste, and easily conserved, and he turns to discuss the question of what is the best use of an able man's time. Prof. Carter at once recognizes the extreme difficulty of finding any satisfactory criteria, quite apart from the extent to which the personal idiosyncrasies of ability must be recognized and given due allowance. The complex nature of intellectual effort does not permit any ready separation of easy or routine tasks and those of greater complexity or strenuous activity. While it is important that the best qualities of mind and experience should be available to deal with difficult, controversial and complicated matters, it is seldom possible to filter out all lesser problems and deal with them at a lower level in the organization. It might be possible to design information systems which could help here in taking care of the lesser problems, but there would remain the problem of how experience is to be gained by the high ability which is coming on; and there is also liable to be disagreement as to function and ability. Prof. B. R. Williams and Prof. Carter found in their industrial studies, in fact, that very little attention had been given to the science of using scientists productively, and he suggests in the present address that in all forms of work which use high ability, there is need for much more study of the conditions which favour effective work. He considers that there are valuable principles to be discovered and reforms to be made.

It is curious, in view of the dearth of material to which Prof. Carter refers, that he makes no reference to the report of the Barlow Committee on Scientific Staff, issued in 1945, which provided one of the best analyses of the conditions for effective scientific work, at least in the Government service, which has yet appeared. He notes, indeed, that men of high ability are often in positions in which they are able to create their own conditions of work, though at the same time he points out that the economic use of brains may involve protecting a man from the self-imposed wastage of his talents, as well as giving him freedom to use them to the full. What needs to be said here, above all, is that it is not so much fresh knowledge about the conditions conducive to effective work—whether it be creative or administrative—at the highest level that is required, but the perceptive application of existing knowledge. So far as the Government is concerned, disregard of what is already known about such conditions is largely responsible for much of the present frustration and wastage, particularly among scientific staffs; and it would seem a sufficiently important matter to warrant attention by the Minister for Science or the Advisory Council for Scientific Policy.

From this aspect of the efficient use of distributed man-power, Prof. Carter turns to discuss the distribution of ability between different occupations. Here he suggests that the determinant factor is the initial choice of occupation, and he stresses the desirability of greater attention to factors which promote mobility between different occupations. Desirable in itself on the grounds of experience, such mobility is a corrective to mistaken or excessive choice of particular occupations, and all the more in that there is still much to learn about factors influencing the initial choice of occupation. Moreover, the choice at present seems often to be needlessly haphazard; it should be possible even with existing knowledge for this choice to be much better informed both in regard to the potentialities of the boy or girl, and the advantages or responsibilities to be found in the occupations to which specialization leads.

The present shortage of mathematics teachers underlines the importance of Prof. Carter's remarks on the distribution of high ability between the universities and elsewhere. Surveys of the employment of graduates made by Political and Economic Planning and other similar surveys have given no full answer to such questions as the distribution of high ability between expanding and declining, or modern and traditional industries, between large and small firms, between different parts of Britain, between the nationalized industries and other parts of the public service. Yet under certain conditions this could be the decisive factor in the success or failure of an industry or a firm; and despite the imperfections of definitions of ability, more research on these matters and on the trends in the demands of various industries and services is needed.

By and large, Prof. Carter outlines very fairly the problems in this field that require attention, although he is inclined to make too little of what could be done with existing knowledge and resources. Curiously enough, while he takes a much less optimistic view as to the existence or extent of the pool of ability on which we can draw than did Prof. G. Seth in addressing Section J (Psychology), he ignores completely, as such, the reservoir of ability to be found in the employment of women which, Prof. Seth notes, could go far to meet any existing shortage of skilled talent. Further, he does not discuss the contribution which improved methods of teaching could make, either in the development of the student or in the more efficient use of the time of the trained teacher. Two points, however, should be noted in conclusion. Prof. Carter fully recognizes that the effective use of trained man-power in the universities is not simply a matter of using the university teacher's time to the best advantage, but is also a part of the national effort in research and the right use of man-power in relation to scarce and expensive scientific equipment —a matter to which also more critical and constructive thought must be given.

Finally, there is the question of what economies of scale may be possible in the use of high ability.

Prof. Carter does not discuss that difficult question in any detail. Indeed, he seems to raise it only to illustrate the complexity of all these issues and how readily they may incline to decisions which are self-frustrating or defeating. It is only too easy to take decisions for one purpose only to find that they have created conditions which destroy the incentives it is sought to provide. We must indeed recognize the existence of these economic problems and give them the study and attention they need; but it cannot be too carefully kept in mind that nothing is easier than to plan out of existence the conditions in which high, and especially creative, ability functions most effectively and efficiently.

PHASES IN EVOLUTION

Grossabläufe der Stammesgeschichte

Erscheinungen und Probleme. Von Prof. Arno Hermann Müller. Pp. vii+116. (Jena: Gustav Fischer Verlag, 1961.) 18.20 D.M.

THE work under review is a revised edition of one originally published in 1955; some of its subject-matter and diagrams have also been incorporated in Vol. 1 of its author's *Lehrbuch der Paläozoologie* (1957). Its purpose is to study the fluctuating fortunes, during their long geological histories, of those major animal groups of which abundant fossil remains are preserved, and to draw such general conclusions as seem possible. The author uses as data the number of genera recorded in successive subdivisions of the geological column, each representing some 15–18 million years. He is not the first palæontologist to have taken up this line of investigation, for an account of very similar work by a group of United States authors has appeared in the *Journal of Paleontology*, **26** (1952). For presentation of the facts he relies mainly on symmetrical diagrams in which the number of genera, grouped according to their time-ranges in differently shaded areas, is plotted horizontally across a median axis representing geological time.

The obvious objection to the choice of genera as the basis of the study is that of differing taxonomic standards among workers who have specialized (as most modern palæontologists tend to do) in fossils belonging to particular families or geological formations; for irresponsible genus splitting might well become interpreted as evidence of unusually intense evolutionary diversification. By taking his data from treatises in which the material has already been digested according to more or less uniform standards the author has probably avoided this pitfall to some extent. In discussing the trilobites he gives two diagrams, based respectively on modern works in which 996 and 1,395 genera are recognized, and he shows that both present the same general picture. In the case of Mollusca other than Cephalopoda his information does not appear to be derived from up-to-date sources. Thus on p. 94 the number of known gastropod genera is given as about 700, whereas in the Wenz–Zilch treatise on this class (1938–60) in the *Handbuch der Paläozoologie* the number of shell-bearing genera recognized is 3,400; the figure 296 given for known genera of Bivalvia (Lamellibranchia) is similarly disproportionately low.

It is probable (although some authors deny it) that the concept of a species is less subjective than that of a genus, so that a census of species would have been a more satisfactory basis for the present study. According to a recent estimate, however, among invertebrates alone the total number of extinct species with hard parts capable of becoming fossilized has been 7–8 millions; and, even though (according to estimates from other sources) those so far described number only from one-hundredth to one-twentieth of the whole, it would clearly have been beyond the capacity of a single worker to assemble the necessary data about them. A count of genera was the only practicable approach to the investigation. One thing is clear; the number of genera and species living at any period does not necessarily bear any relation to the total of adult individuals belonging to a group or give any indication of its relative competitive success. The former is incalculable, the latter unassessable.

In the work under review the diagrams already mentioned show a succession of bulges and contractions; not one is a steadily broadening triangle, although there is some approach to this with the Gastropoda and Bivalvia. A marked bulge corresponds to what the author terms a "Virenzphase", a phase of profuse mutational changes. The most abrupt and intense of such phases are the 'explosive' or 'eruptive bursts' of United States and British writers. According to the author, three types of evolutionary history are distinguishable. In the "regressive" type (Trilobita, Graptolithina, Nautiloidea) there is a series of "Virenzphasen" decreasing in intensity and duration and leading eventually to extinction. In the "progressive" type there is a succession of such phases increasing in intensity and duration, although ultimately the group may fail to survive a subsequent phase of shrinkage and crisis. (For example, during the two intervals, at the close of the Permian and of the Triassic periods respectively, between their three "Virenzphasen" the Ammonoidea almost died out, but two or three genera just managed to survive in each case; a third crisis at the end of the Cretaceous, however, sealed the fate of the whole order.) In the third type of evolutionary history, the "intermediate" type (Brachiopoda, Amphibia), there is a series of complex "Virenzphasen" each consisting of a relatively weak initial phase, an intense main phase, and a weak late phase.

Applying these results to groups now living, the author considers the Nautiloidea, reduced to a single genus following what he believes to be the final phase of a history of the "regressive" type, to be probably well on the way to extinction. The Echinoidea and Reptilia, however, although now on the decline, would appear to have further "Virenzphasen" ahead. The Brachiopoda may pass through a weak phase of increase before their final extinction. The Mammalia are now passing through the main phase of a "progressive" series; at least, they were doing so before the advent of man.

Chapters are devoted to a survey of knowledge of pre-Cambrian life (illustrations of recent finds in South Australia are reproduced in some of the plates) and to a discussion of the various factors which may have combined to determine the history of life through the ages. A study of the diagrams in this book suggests to me that the author's three-fold classification is an over-simplification. The future, in fact, seems to be entirely unpredictable. L. R. Cox

XUM

CELLS IN EVOLUTION

Cytology and Evolution

By Dr. E. N. Willmer. Pp. x+430. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1960.) 70*s*.

THIS is a scholarly work, for Dr. Willmer has given thought to his subject for many years. Inevitably, it is not an easy book to read and assimilate. *Cytology and Evolution* is a challenging title ; at one extreme, it is concerned with the techniques required to reveal the nature of cellular physiology, and at the other, the infinitely complicated problem of cell evolution in higher organisms. Characteristically, Dr. Willmer introduces the subject by visualizing "the conversion of the Victorian carriage into the racing car of to-day" as a lesson in evolution. He is intrigued by "the mystery of a common organization", and his argument opens by examining the similarity in the fine structure of cilia throughout the lower and higher forms of life.

The author knows as much as anyone could about the behaviour of cells isolated from their normal environment and grown in tissue culture. From his experience, he has developed the interesting simplification that tissue culture encourages the growth of three stem-lines of cells. These are the epitheliocytes, the mechanocytes, and the amœbocytes, and the photographs selected are most effective in illustrating his point of view.

The book begins its own organic growth with the definition of these different cell forms, and here the reader meets the first complications in the argument. What, the author has asked himself, are the morphological and physiological features which define an epitheliocyte, a mechanocyte, and an amœbocyte? Many examples can be cited which apparently define or separate one cell from another, such as the strange phenomenon that an epitheliocyte will be unaffected by the death of its neighbour (punctured through his nucleus), while a fibroblastic cell will choose to die along with its neighbouring cell. There is also the problem of the permanent cell lines which have been maintained for many years in tissue culture and which appear to have become a 'classless' society.

The basic ideas of the author take up one-third of the book ; their effect on the analysis of embryogenesis is developed in the remaining two-thirds of the book. Dr. Willmer takes the reader as gently as possible through the reversible transformation of flagellate cell types to amœboid cell types, as found in certain lower organisms, particularly *Naegleria*, on which he has himself done fascinating work. One reader, at least, is concerned that he has chosen only to discuss the transformations in terms of homoiostasis, and does not envisage, for example, the possibility that the ionic environment might alter the templates for enzyme formation within the cell.

In developing his theme, Dr. Willmer seems at first to be 'Lamarckian' rather than 'Darwinian' in his outlook. He instances the fact that "many animals independently under Arctic conditions cease to produce melanin in their skins, and so all become white", and evidently considers this to be an adaptation to the iciness of their environment instead of natural selection. How else can one construe the observation on the same page (227) that "when phenyl-alanine is added to tissue cultures of even the ventral ectoderm, the cells of which would never normally produce melanin, some of these cells can actually be induced to do so"? Perhaps it is only a lapse in analogy, for clearly the author favours natural selection when he later describes the disadvantages of "poor form-vision under critical conditions of hunting or being hunted".

In discussing the development of the kidneys, gonads, adrenal cortex, and other cell populations closely related to them, he envisages their origin from the cœlomoducts and the nephridia of the more primitive invertebrates. This last third of the book is a mine of valuable information hidden in catacombs of unresolved definition and delineation. When, for example, at page 274, the reader finds that the nephron of kidney tissue would best be described as "some sort of nephromixium", he later has to try to understand why the spermatic tubules and their mesonephric elements should be described as a "mixonephridium".

Appropriately the book ends on the subject of light and evolution of vision, and the final chapter is an illuminating summary of the author's concepts of evolutionary cytology. Here, Dr. Willmer states concisely the principles on which he bases his view about the evolution of cell types and embryogenesis. As a cytologist he treats cell structure with reverence and appears to be horrified by the butchery involved in the biochemical analysis of sub-cellular fractions. Nevertheless, the book concludes that there is an exciting 'jungle pathway' ahead for microbiochemistry and cellular physiology. Dr. Willmer has done a great service by creating a new and well-indexed route through the jungle of cytological embryology.

I. Leslie

A SURVEY OF EXTRA-SENSORY PERCEPTION

The Hidden Springs

An Enquiry into Extra-Sensory Perception. By Renée Haynes. Pp. 264+9 plates. (London : Hollis and Carter, Ltd., 1961.) 30*s*. net.

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E. J. DINGWALL

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Klimadiagramm-Weltatlas

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G. MANLEY

HISTOLOGY AND ELECTRON MICROSCOPY

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The treatment is clear and thorough, being illustrated with photographs of various operations, as well as with typical micrographs. The author is at

CELLS IN EVOLUTION

Cytology and Evolution

By Dr. E. N. Willmer. Pp. x+430. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 70*s*.

THIS is a scholarly work, for Dr. Willmer has given thought to his subject for many years. Inevitably, it is not an easy book to read and assimilate. *Cytology and Evolution* is a challenging title; at one extreme, it is concerned with the techniques required to reveal the nature of cellular physiology, and at the other, the infinitely complicated problem of cell evolution in higher organisms. Characteristically, Dr. Willmer introduces the subject by visualizing "the conversion of the Victorian carriage into the racing car of to-day" as a lesson in evolution. He is intrigued by "the mystery of a common organization", and his argument opens by examining the similarity in the fine structure of cilia throughout the lower and higher forms of life.

The author knows as much as anyone could about the behaviour of cells isolated from their normal environment and grown in tissue culture. From his experience, he has developed the interesting simplification that tissue culture encourages the growth of three stem-lines of cells. These are the epitheliocytes, the mechanocytes, and the amœbocytes, and the photographs selected are most effective in illustrating his point of view.

The book begins its own organic growth with the definition of these different cell forms, and here the reader meets the first complications in the argument. What, the author has asked himself, are the morphological and physiological features which define an epitheliocyte, a mechanocyte, and an amœbocyte? Many examples can be cited which apparently define or separate one cell from another, such as the strange phenomenon that an epitheliocyte will be unaffected by the death of its neighbour (punctured through his nucleus), while a fibroblastic cell will choose to die along with its neighbouring cell. There is also the problem of the permanent cell lines which have been maintained for many years in tissue culture and which appear to have become a 'classless' society.

The basic ideas of the author take up one-third of the book; their effect on the analysis of embryogenesis is developed in the remaining two-thirds of the book. Dr. Willmer takes the reader as gently as possible through the reversible transformation of flagellate cell types to amœboid cell types, as found in certain lower organisms, particularly *Naegleria*, on which he has himself done fascinating work. One reader, at least, is concerned that he has chosen only to discuss the transformations in terms of homoiostasis, and does not envisage, for example, the possibility that the ionic environment might alter the templates for enzyme formation within the cell.

In developing his theme, Dr. Willmer seems at first to be 'Lamarckian' rather than 'Darwinian' in his outlook. He instances the fact that "many animals independently under Arctic conditions cease to produce melanin in their skins, and so all become white", and evidently considers this to be an adaptation to the iciness of their environment instead of natural selection. How else can one construe the observation on the same page (227) that "when phenyl-alanine is added to tissue cultures of even the ventral ectoderm, the cells of which would never normally produce melanin, some of these cells can actually be induced to do so"? Perhaps it is only a lapse in analogy, for clearly the author favours natural selection when he later describes the disadvantages of "poor form-vision under critical conditions of hunting or being hunted".

In discussing the development of the kidneys, gonads, adrenal cortex, and other cell populations closely related to them, he envisages their origin from the cœlomoducts and the nephridia of the more primitive invertebrates. This last third of the book is a mine of valuable information hidden in catacombs of unresolved definition and delineation. When, for example, at page 274, the reader finds that the nephron of kidney tissue would best be described as "some sort of nephromixium", he later has to try to understand why the spermatic tubules and their mesonephric elements should be described as a "mixonephridium".

Appropriately the book ends on the subject of light and evolution of vision, and the final chapter is an illuminating summary of the author's concepts of evolutionary cytology. Here, Dr. Willmer states concisely the principles on which he bases his view about the evolution of cell types and embryogenesis. As a cytologist he treats cell structure with reverence and appears to be horrified by the butchery involved in the biochemical analysis of sub-cellular fractions. Nevertheless, the book concludes that there is an exciting 'jungle pathway' ahead for microbiochemistry and cellular physiology. Dr. Willmer has done a great service by creating a new and well-indexed route through the jungle of cytological embryology.

I. Leslie

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to describe many "seemingly small, but actually critical details" of preparation. This is a praiseworthy aim, but it opens the way to dogmatism and the glossing over of differences of opinion between rival schools of thought. However, the author is usually frank in stating where his own experience has been limited, and he also provides a list of references at the end of each chapter, so that the novice can seek alternative advice. In the almost complete absence of regular courses of instruction in the techniques of electron microscopy, the present text provides as comprehensive a handbook as can be expected from a single pen. It is designed to be used at the bench rather than in the library, and should be beside every electron microscope in use in a biological or medical laboratory.

V. E. Cosslett

RADIATION PROTECTION

Radiation Protection and Recovery

Edited by Alexander Hollaender. (International Series of Monographs on Pure and Applied Biology. Division : Modern Trends in Physiological Sciences. Vol. 7.) Pp. v+392. (London and New York : Pergamon Press, 1960.) 70*s*. net.

Problems of Infection, Immunity and Allergy in Acute Radiation Diseases

By N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov and V. F. Sosova. Translated from the Russian by Lydia Venters. Translation edited by R. Clarke. Pp. viii+165. (London and New York : Pergamon Press, 1961.) 50*s*.

The Delayed Effects of Whole-Body Radiation

A Symposium. Edited by Bernard B. Watson. (Jointly sponsored by the Operations Research Office and the Walter Reed Army Institute of Research.) Pp. ix+80. (Baltimore, Md. : The Johns Hopkins Press ; London : Oxford University Press, 1960.) 36*s*. net.

The Entry of Fission Products into Food Chains

Edited by J. F. Loutit and R. Scott Russell. (Progress in Nuclear Energy. Series 6 : Biological Sciences, Vol. 3.) Pp. vii+154+xii. (London and New York : Pergamon Press, 1961.) 45*s*. net.

Radioactivity for Pharmaceutical and Allied Research Laboratories

Edited by Abraham Edelmann. (A Symposium sponsored by Nuclear Sciences and Engineering Corporation, held in Uniontown, Pennsylvania.) Pp. xii+171. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1960.) 6 dollars.

CURRENT and forthcoming legislation, in the form of Factories Acts such as the "Ionising Radiations (Sealed Sources) Regulations 1961", and its companion dealing with the use of unsealed radioactive substances in industry, require the appointment of a medical officer, who will carry out examinations on those workers exposed to radioactive materials. Much of this work will fall on the industrial medical officer already connected with the firm, or the local medical officer of health, but it will be extremely unusual to find one with any knowledge on the effects of radiation. It is true that a set of notes (*Medical Supervision in Radiation Work*) has been drawn up by the World Health Organization, but the keen medical officer will generally wish to supplement this knowledge with information on the effects of relatively high doses or chronic exposure to relatively low doses of radiation.

The subject is, however, still in its infancy, and much of the information is contained only in professional journals or proceedings of learned societies. The production of any text-book on the subject is therefore of considerable interest at the present time, though from this point of view the books under review are rather too specialized for the average medical officer. On the other hand, there is much to be learned from them, even by the non-specialized reader, and while the first three are primarily medical in character, the health physicist can also find much to interest him here.

All five books are the products of a team of authors, each a specialist in some particular part of the field.

Probably the most important is *Radiation Protection and Recovery*. This is not a comprehensive text-book, rightly concentrating on biological, physiological and biochemical methods of protection and leaving discussion of shielding, etc., to physicists. Its stated aim is to present some of the background of a rapidly developing subject, and in this it succeeds. A considerable amount of space is devoted to experimental treatment of acute whole-body radiation injury in mammals and to the allied problem of antibody formation, which is of great importance in connexion with bone-marrow implantation.

A remarkably extensive bibliography of some 1,500 references is included ; this alone should make the book a valuable source of information in this field.

The second book is a translation, sponsored by the U.S. Atomic Energy Commission, of a monograph published in Moscow in 1958 and is probably not, therefore, fully representative of current thought in the Soviet Union. It deals exclusively with the results of animal experiments on auto-infection following irradiation, natural and induced immunization, allergic reactivity, and infectious diseases in radiation sickness ; again there is an extensive bibliography, about half the 326 references being non-Russian in origin.

From the research worker's point of view, *The Delayed Effects of Whole-body Radiation* will be disappointing, though for those generally concerned with radiation protection there is some interesting material. Effects on life-span, the induction of malignant diseases and the production of cataracts are each covered in two chapters, one on animals and the other on man ; genetic aspects are treated jointly.

The Entry of Fission Products into Food Chains is not directly comparable with the previous three books, but it is justifiable to include it in this review since the subject is so closely connected with radiation protection. As one might expect of the editors, who are authorities in this field, we have here a most informative group of papers, which were originally produced as reports by the Atomic Weapons Research Establishment. There are two sections on the retention of fall-out on herbage and ripe cereals, but the majority of the papers deal with animal experiments connected with actual nuclear weapon tests. This is definitely a specialist's book, but should also be of great interest to anyone concerned with the wider aspects of radiation protection and civil defence.

The last book is unfortunately titled. It is certainly not a text-book for pharmaceutical laboratories, being simply the proceedings of a symposium ; it is very mixed in character and is too varied in standard to be of great interest.

Hugh D. Evans

Structural Concrete

By Prof. Kurt Billig. Pp. xx+1020. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1960.) 84*s*. net.

PROF. BILLIG is a well-known author of books and papers on reinforced concrete and prestressed concrete. His present volume aims to provide a "minor encyclopædia on structural concrete", and this is indeed a fair description of the work. It is divided into three sections, the first dealing with materials, the second with structural design and the third with design and construction. In attempting to cover such a wide field in a single volume of manageable size the author is inevitably faced with very difficult problems of selection and condensation. On the whole, Prof. Billig has tackled these problems successfully although the depth of treatment varies somewhat from one chapter to another.

Part 1 is devoted mainly to concrete technology, that is to a discussion of the properties, selection and testing of cements and aggregates and the techniques of mixing, placing and consolidating concrete. A chapter is devoted to the important question of durability. Part 2 considers first the basis of structural design in concrete and goes on to discuss in considerable detail the methods of proportioning practically every kind of structural element in reinforced concrete, including beams, columns, slabs, arches and shells. A great amount of ground is covered, and numerous diagrams, tables and examples are included. The third section of the book discusses the use of structural concrete for a wide variety of building and engineering structures, with reference to actual examples.

A very good balance has been achieved between structural analysis, practical design considerations and concrete practice. The book is a veritable mine of information for structural engineers and architects, to whom it can be highly recommended.

A. W. HENDRY

An Electronic Outline of Organic Chemistry

By Dr. S. Horwood Tucker. Pp. xvi+478. (London: University of London Press, Ltd., 1959.) 63*s*. net.

THIS is a book, written for undergraduates, in which the reactions of the commoner types of organic compounds are interpreted in terms of the electronic theory. Dr. Tucker is to be congratulated for having rescued so effectively the "gems of electronic exposition" from submergence in the "heavy matrix of comprehensive fact". Not that the importance of experimental fact is in any way diminished: indeed, just sufficient significant facts have been carefully selected and lucidly expounded to present to the undergraduate a clear and reasonable account of the essentials of organic chemical theory.

There are, however, certain omissions which, though they do not detract from the overall value of the book, nevertheless seem surprising, especially since these topics are now included in undergraduate lecture courses. Thus, the reactions of benzyne, and of carbenes (even in connexion with the Reimer–Tiemann reaction, p. 407) are neglected; likewise Bender's work on ester hydrolysis receives no mention.

The text is well supplied with references to both individual papers and review articles.

It is a pity that several misprints remained undetected in the proofs. Fortunately, most of them are relatively trivial and they do not seriously affect the sense of the text.

The book is well produced and possesses a good index.

While this book can be strongly recommended to students, it is likely that its price will deter all but the most affluent, or the most self-denying of the impecunious, from purchasing it. NEVILLE H. P. SMITH

Proceedings of the 1960 Annual International Conference on High Energy Physics at Rochester

N.Y., August 25–September 1, 1960. Edited by E. C. G. Sudarshan, J. H. Tinlot, and A. C. Melissinos. Pp. xxv+890. (Rochester, N.Y.: University of Rochester, 1960. Distributed by Interscience Publishers, Inc., New York and London.) 13.50 dollars.

THE International Conference on High Energy Physics which was held in Rochester, N.Y., was the tenth in the series. This annual conference is acknowledged to be the most important one in this field. Its purpose is to assemble a representative group of workers from high-energy laboratories throughout the world to discuss the experimental results obtained and the theoretical developments which have occurred during the previous year.

The *Proceedings* are divided into three sections. The first section covers the initial "working" or "S" sessions, several of which were usually held concurrently. The second, "R" section, contains the reports of the *rapporteurs*. Finally, in the last three chapters are contained, in full, the papers presented at three "plenary" sessions. The subject-matter of the conference was limited to the following topics: the strong interactions of pions and nucleons; the strong interactions of strange particles; weak interactions; the structure of elementary particles; new results at super-high energies, and finally, theories of elementary particles.

The editors, secretaries and all concerned with the publication of the *Proceedings* deserve the highest praise for producing such a magnificent record within two months of the end of the Conference. No physicist interested in elementary particle physics can afford to be without the volume close at hand.

G. H. STAFFORD

Probability Theory

By Prof. Michel Loève. Second edition. (The University Series in Higher Mathematics.) Pp. xvi+685. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 111*s*.

THE first edition of this book appeared in 1955 and went a long way to fill a serious gap in the mathematical literature. The new edition, revised in detail and longer by two chapters, comes a little nearer to closing the gap and is still the only (and an excellent) approximation to a comprehensive textbook on mathematical probability. The new chapter 11 (64 pages) deals with the generalities of the theory of stochastic processes (including separability and measurability), continuous-parameter martingales, and decomposability theorems. The new chapter 12 (95 pages) deals with Markov processes. It starts with an account of the 'strong' Markov property, and passes on to a discussion of the differentiability of transition functions before taking up the theory of one-parameter semi-groups of operators, which it pursues as far as a proof of the basic Hille–Yosida theorem. Finally, there is a valuable synthesis of the recent work by Feller, Dynkin and others on diffusion theory. D. G. KENDALL

PROF. A. J. EAMES AND THE MORPHOLOGY OF THE ANGIOSPERMS

By Prof. F. C. STEWARD, F.R.S.
Cornell University

BY a happy combination of circumstances, the McGraw Hill Publications in the Botanical Sciences have issued *Morphology of the Angiosperms*, by emeritus professor of botany, Arthur J. Eames, in the author's eightieth year*. It is therefore fitting that botanists should congratulate Prof. Eames on October 10, his eightieth birthday, on an active and fruitful life devoted to the study of plants and to the teaching of botany. His latest book, which is a crowning achievement in an already distinguished career, is also a proper subject for congratulation.

For almost fifty years (since 1912) Prof. Eames has been identified with botany at Cornell University. He joined the staff of that University, fresh from his first travels to Australia and New Zealand; and, when botany was transferred wholly from the College of Arts and Sciences to the College of Agriculture in 1913, Prof. Eames formed one of that group which was to make the newly constituted Cornell Botany Department known and respected the world over by the mid-1920's and '30's. Now, in his eightieth year, Prof. Eames is the only one of that first group who is still actively engaged in botanical research on the Cornell campus. Throughout that near half-century, his presence has lent distinction to Cornell botany, and his work has enriched the subject everywhere. This distinction was recognized by the University of Glasgow in 1956 by the award of an honorary LL.D. degree.

Although Prof. Eames retired from active teaching in 1949, he has led a full life since, and the title 'emeritus professor' has been to him a challenge and an opportunity to pursue further scholarly work, of which the *Morphology of the Angiosperms* is a product. Never was the title 'emeritus professor' more richly deserved, nor more gracefully borne, for throughout this period of retirement he has set an example of continued dedication to his lifelong study of plants. He has also been ever ready to share his knowledge, whether in the casual conference or the country walks in which his love of Nature excels. These brief congratulations would, however, be incomplete—and even inappropriate—without reference to Mrs. Eames, who was herself technically trained in botany and has been a great help to Prof. Eames throughout, and especially so in the laborious task of producing this latest book.

It is appropriate at this point to look back on Prof. Eames's career and on his influence on teaching and research in botany. Together with the late Prof. Wiegand, he produced the *Flora of the Cayuga Lake Basin*—in the obvious desire to know first the plants which were closest at hand. Indeed, many of the early collections which formed the basis of the present Wiegand herbarium were a part of this relationship. Although morphology has always been his consuming interest, Prof. Eames first became most widely known for the *Introduction to Plant Anatomy*, first published in 1925 and revised in 1947. This book, compiled with his first graduate student, Prof. McDaniels (also now an emeritus professor) was, and in many places still is, the essential modern text on which successive generations of botanical students have become aware of plant anatomy. Those who, in the 1920's and 1930's, were privileged to take Eames's course in plant anatomy know that much of the wealth of illustrative material in the form of prepared slides and sections had actually been produced by Eames and McDaniels. It was a rich experience for the student to see not merely a stem, or one example of this or that anatomical feature or tissue, but a rich array of representative examples. In this, as indeed in the study of morphology, Prof. Eames's outlook was always comprehensive. Even then, however, the book on anatomy was essentially the background, or working manual; for it had distilled the essence of Eames's experience of anatomy, and his lectures clothed this framework with much more that had been gained from a rich observational experience. Yet, with this far-ranging interest to comprehend the plant kingdom as a whole, Prof. Eames has in all his books shown that rare ability to state the essentials, to distil it down to its minimum, so that often a paragraph presupposes the equivalent of a research investigation before it could be written.

The new *Morphology of the Angiosperms* is again in this style, though necessarily it is a larger work than its predecessor on the *Morphology of Lower Vascular Plants*, which was published in 1936. The new book comprises 518 pages, 147 well-chosen figures, with often several pages of selected references at the end of each chapter. The *Morphology of the Angiosperms* is again remarkable for its concise style and for the way its author has been able to compress the knowledge gained from the literature and his own experience. Again one senses that, over the years in which this volume has been prepared, Prof. Eames had conducted the equivalent of many research projects, each worthy of separate review, on subject-matter which in the book is concisely treated.

Prof. Eames's first book on morphology dealt with the lower vascular plants. In respose to an evident need, however, the new work deals with the angiosperms—the largest in number, most complex and most important vascular plants. One wonders whether, and even hopes that, Prof. Eames will now fill the gap and enrich botanical science by yet another work on the gymnosperms.

Characteristically, Prof. Eames does not in his introduction enlarge on what morphology is, and in these days of ever-narrowing specialization—of plant physiology and biochemistry; of cytology and genetics; of cell physiology, cytochemistry and ultrastructure; of enzymology and molecular biology—some may even ask, what is it all about?

The book is simply and completely about angiosperms—the largest modern group of seed plants: and morphology here implies strictly that the work deals with the structure and the form of these plants. The work distils from the literature and from Prof.

* *Morphology of the Angiosperms.* By Prof. Arthur J. Eames. (McGraw-Hill Publications in the Botanical Sciences.) Pp. xiii + 518. (New York: McGraw-Hill Book Co., Inc.; London: McGraw-Hill Publishing Co., Ltd., 1961.) 104*s.* 6*d.*

Eames's own rich experience what one can interpret about angiosperms from what one observes of them, and observation here means visual observation, aided by the lens and the optical microscope. Among the allied disciplines that are invoked to interpret angiosperms from this point of view, physiology, biochemistry, genetics, ultra- and sub-microscopic structure as yet find no place. Prof. Eames looks at plants as they are, as he sees them, and by an essentially comparative approach seeks information which is to be interpreted against the background of evolution and phylogeny. In this sense the book follows a classical tradition.

But it is precisely because so much modern biology and botany has become so dissected into specialties that this comprehensive work is now so valuable. Indeed, it should be especially valued even by those students of plants whose interests lead them to experimentation, to culturing organs and cells, to the study of biochemistry and nutrition, and to the approach to truth at the molecular level. A generation which is steeped in this sort of thing has rarely had the time, even if it had the interest, to acquire the breadth of knowledge and scholarship about plants that Prof. Eames's book now contains. Even a glance at the table of contents, for the headings and sub-headings of the chapters, may be a salutary reminder to some that although biological events may often be determined at the cellular and sub-cellular level, they finally culminate in organization that needs to be comprehended at much higher levels. Those of us who attempt to exploit the new tools of physiology, biochemistry and ultra-structure need particularly to appreciate the nature and the relationships of the cells and organs with which we work.

Here then is a modern work which synthesizes knowledge in a comparative way about such parts of the plant body as shoots and roots, leaves and stipules; nodes and branches; the inflorescence and the flower; the androecium, the stamens and pollen; the gynœcium, carpel and ovule; sporogenesis, fertilization, embryology; seeds and fruits, etc.; and, in the course of this treatment, it introduces the reader succinctly to classical morphological concepts and to the author's contemporary views. But it is in the chapters on the stamen and the carpel and their derivation from foliar organs that Prof. Eames's personal interest and interpretations are particularly evident.

Inevitably this work, which could not possibly be summarized here, will find a sure place among the botanical classics, and it will necessarily be read by those who make morphology their speciality. But this book should do more than this, for it should be a ready means to guide those who work experimentally with angiosperms, and especially those concerned with problems of growth and reproduction, into the proper interpretation of the nature of the material with which they deal. There is a dangerous and mistaken idea abroad that biology has outgrown the older descriptive disciplines of morphology, anatomy and taxonomy. Prof. Eames's book should dispel this idea by bringing a fresh presentation of this older subject. In fact one notes, parenthetically, that Prof. Eames finds that the older literature is often nearer the truth than much which has been written more recently.

A less-modest man than Prof. Arthur J. Eames might, in his eightieth year, have titled his book "The Angiosperms as I see them", for he is one of the very few who have had the experience and have the knowledge, the vision and the understanding for this great task.

Botanists everywhere will feel indebted to him for this additional achievement in a long and fruitful career.

RE-EVALUATION OF SOME FACTORS IN ARTERIOSCLEROSIS

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MORPHOLOGICALLY, arteriosclerosis is defined as a disease of the intimal layers of the arterial wall occurring focally and associated with intimal thickening and lipid deposition. The earliest changes can be seen in infants. The internal elastic membrane becomes deformed, calcified, and fibrotic. These foci are eventually called atheromata. Disturbances of the flow of blood may ensue. Frequent sites of these changes are, in addition to the aorta, the coronary, cerebral, and lower leg arteries. Any theory of the disease will have to account for these facts at least.

Thickened arteries (παχειη αρτερίη) were noted nearly two thousand years ago by Aretaeus of Cappadocia. The term artheromatous (or tuberculous) was used by Morgagni[1] and perhaps even by others before him, but modern ideas about the disease were developed by the great French physicians of the Napoleonic era. Lobstein[2] classified the disease as a nosological entity between 1820 and 1833 and was the first to use the name arteriosclerosis. He had the first inkling that the disease may be related to cerebral accidents. The relation of myocardial infarction to arteriosclerosis was established much later. After the great English physicians of the eighteenth century had recognized the association of coronary disease and angina pectoris, it took another century to establish that this coronary disease is arteriosclerosis.

Soon after Lobstein's description, two theories as to the pathogenesis of the disease were developed which still dominate our thinking. Rokitansky thought that abnormal clotting processes, resulting secondarily in the organization of thrombi, were responsible for the arterial roughness[3]. Virchow assumed that, in what he called endarteritis chronica deformans[4], focal inflammatory changes are followed by deposition of lipids such as cholesterol, which had just been discovered. Virchow may have been somewhat handicapped by his inability to distinguish arteriosclerosis from other arterial diseases, such as syphilitic aortitis, which became possible only at the end of the nineteeth century.

In addition to syphilitic aortitis, other arterial diseases involving calcification were recognized, particularly media necrosis[5]. Whether or not the latter is at all related to arteriosclerosis is by no means

sure. For purposes of definition it should, at the present time, be excluded. This would make unnecessary the use of such terms as atherosclerosis, angiosclerosis, and the like.

At about the turn of the century, it was discovered that early arteriosclerotic lesions occur in children[6], and this observation has since been extended to infants[7]. However, at the same time, the rapid progress of biochemistry and nutrition began to influence the work on arteriosclerosis[8], so that, since then, most investigations have centred around the disturbances of lipid metabolism and their association with dietary fat. It is curious how little attention has been paid to the early occurrence of the lesions.

By the end of the nineteenth century, it had been well established that arteriosclerosis, in common with other systemic diseases, is subject to modifications by a number of general factors.

The disease runs in families, and probably has a genetic factor. Sex plays a part in that women below the age of fifty have fewer heart attacks than men. Physical activity is also a factor because people with sedentary work show extensive lesions more frequently than people doing manual work. Arterial hypertension is associated with more advanced lesions. Arteriosclerosis occurs more extensively in old persons. The abuse of coffee and tobacco leads to more frequent myocardial infarctions, whereas the incidence in heavy drinkers is relatively low. The stress that those in managerial positions are subjected to has been cited, although not completely convincingly, as contributing to a high incidence among this group.

The accummulation of fatty substances in the atheroma suggested examination of lipid metabolism. At about the turn of the century, it was found that patients afflicted with advanced arteriosclerosis have lipæmic sera[9], and it was soon established that the serum cholesterol is frequently elevated[10]. Further work elucidated other disturbances of lipid metabolism in arteriosclerosis. Important is the elevation of the β-lipoprotein fraction of the serum[11]; it contains most of the cholesterol, two-thirds of which is esterified with oleic and linoleic acids[12]. Finally, it was discovered that, in advanced arteriosclerosis, the saturated and oleic acid-levels of the serum are often elevated, whereas the polyunsaturated levels are lower than normal.

When these lipid disturbances were first examined, investigators considered them as the consequence rather than the cause of the lesion; and it is certainly true that many chronic diseases are accompanied by similar lipid changes. However, since the fateful experiments with cholesterol feeding, which were interpreted as proof that cholesterol produces arteriosclerosis in animals[13], many leading investigators have been inclined, during the past fifty years, to accept disturbances of lipid metabolism as etiological factors in arteriosclerosis.

If one were to summarize the evidence implicating cholesterol (or perhaps the β-lipoprotein fraction), one might choose these: animal experiments involving the feeding of large amounts of cholesterol or the induction of an abnormal rise in serum cholesterol; its presence in large amounts in the atheroma; higher serum cholesterol-levels in diseases predisposing people to arteriosclerosis (hypothyroidism, nephrosis, familial hypercholesterolæmia); and the fact that its increase in arteriosclerosis patients permits some degree of predictability as to the state of the disease.

With regard to animal experiments, it has frequently been pointed out that the high cholesterol diets which induce lesions having a high cholesterol content are entirely unphysiological. One finds that cholesterol is deposited in most organs[14]; but it is usually the blood vessels which are singled out for discussion. It has been argued by some pathologists that the morphology of the lesions bears little resemblance to arteriosclerosis[15] and that the disease scarcely ever gives rise to myocardial infarctions. A number of experiments has been described in which arterial disease associated with cholesterol deposits has been produced on cholesterol-free diets[16], whereas, in others, the serum cholesterol-level was raised to ten times its normal value without producing arterial lesions. Therefore, at the present time it does not seem that the lesions induced in animals by cholesterol feeding are analogous to human arteriosclerosis.

In a discussion of the presence of cholesterol in the lesions, it is well to recall some general facts about cholesterol metabolism. All organs, and especially the liver, produce much more cholesterol than a human being would eat in any reasonable diet, and feeding of as much as 10 gm. of crystalline cholesterol (compared with about 500 mgm. in a normal diet) does not influence the serum cholesterol-level in humans (it does in animals). Of the total cholesterol produced by the body, about 2 per cent is known to be used in the synthesis of steroid hormones. The function of the rest is largely unknown. One may speculate that, under normal conditions, it forms a building material for all cells[17]; and this guess is supported by its role in disease. As I have stated before, disturbances of lipid metabolism like those in arteriosclerosis occur in many chronic diseases. In tuberculosis, for example, the onset of the disease is followed by hypercholesterolæmia and the deposition of large amounts of cholesterol in the tubercle, which, in the lungs, may afford some protection against cavitation. These changes in cholesterol metabolism can be considered as a defence mechanism of the body. Similar mechanisms are seen in many other chronic diseases (nephrotic kidneys, thyroid tumours, uterine fibroids, old scars). Sometimes, the inflammatory process leads to such an accummulation of cholesterol that the repair process becomes a danger, as with gall stones and kidney stones or when cholesterol crystals break off an arteriosclerotic lesion and produce an embolism[18]. If one assumes that the pathology of cholesterol metabolism in arteriosclerosis is similar to that in other chronic diseases, one could interpret the presence of cholesterol in the lesions as a process of repair.

Such a hypothesis could also explain the premature arteriosclerosis associated with chronic diseases such as nephrosis and hypothyroidism. These conditions are associated with generalized tissue damage which, in turn, evokes a generalized repair process, as in severe rheumatic conditions or in syphilis; this repair process may involve the arterial lesions which are present in all of us to some extent from infancy. Thus, one may also understand the predictive value of elevated serum cholesterol-levels in arteriosclerosis and even of the apparently anomalous low levels seen in some advanced cases[19]. Furthermore, one may speculate that therapeutic reduction of serum cholesterol may have its disadvantages unless one is sure that the lower level in serum does not reflect some damage to the animal. This is not such a remote possibility in view of the fact that feeding of oxidized

fats which are toxic leads to a reduction in serum cholesterol[20].

Because arteriosclerosis, in Western countries, is now the main cause of death, investigation of life expectancy can in a general way be interpreted as work on arteriosclerosis. The fact that obese people have a shorter life expectancy needs no elaboration. Careful autopsy studies immediately after the lean years of the two World Wars in Vienna and Norway respectively showed a remarkable decrease in the incidence and severity of arteriosclerosis, which manifested itself in fewer strokes and heart attacks[21]. There was a rapid increase as soon as the food intake returned to normal. This is at the bottom of all speculations about the relation of diet to arteriosclerosis.

Dietary fats have been given tremendous importance in the consideration of arteriosclerosis. They exert a two-fold influence. Unspecifically, they are the densest source of calories and their feeding reduces energy requirements which increases body-weight and arteriosclerosis. But more specifically, they wield a strong influence on serum cholesterol unrelated to their own cholesterol content. It has been claimed that the number of heart attacks is a function of the fat content of the diet ; Japan, with a dietary fat-level of about 8 per cent, has the fewest, and the United States, with 40 per cent of fat in the diet, heads the list. The incidence among Japanese living in California and eating the same diet as the rest of the population is the same as for the rest of the United States[22].

This hypothesis has not remained uncontested. It has been pointed out that the incidence of strokes in Japan is higher than in the United States and that arteriosclerosis is less of a problem in Thailand than in Japan despite a much higher dietary fat-level[23]. Similarly, it has been claimed that there is no difference in the incidence of arteriosclerosis in London and Calcutta despite widely differing diets[24]. Furthermore, it has been shown that, among those countries having a daily fat intake of about 120 gm., the rate of myocardial infarction is four times as high in the United States as in the Netherlands[25]. In general, however, there is fair agreement that a very low fat intake, especially in association with a low total caloric intake, leads to fewer myocardial infarctions. With regard to strokes, it is difficult to evaluate the data.

Human life-time studies do not exist, and animal experiments are sparse and contradictory with regard to the level and kind of fat eaten. Therefore, the main argument as to the advisability of eating specific fats rests on their influence on serum cholesterol, and possibly also on blood coagulation. From the point of view of the tenuous hypothesis that serum cholesterol-levels may reflect the extent of tissue damage and in view of the over-all predictive value of these levels, the question of whether manipulation of serum-levels by fat is meaningful is of interest ; but ardent public discussion is, at best, premature. Much has been made of animal versus vegetable fats[26,27], saturation versus unsaturation[28], and linoleic acid deficiency[29]. The fact that the saturated long-chain fraction of coconut oil increases and the saturated medium chain fraction decreases serum cholesterol-levels[30,31] is one of the many examples that no generalization can be made and that fats have to be considered individually. Only the theory that liquid fats have some advantages over hard ones has received some experimental support.

The discovery of heparin and the 'spoiled sweet clover disease' opened the way for an important advance in the treatment of arteriosclerosis. Based on Rokitansky's hypothesis as to the importance of thrombus formation, the newly developed anti-coagulants have been used successfully in preventing many heart attacks and strokes. This approach was almost immediately influenced by prevailing ideas about the role of fat in arteriosclerosis, and a large number of papers has been written dealing with the question of whether the lipæmia occurring after the intake of fat increases the tendency of the blood to clot. Although, with respect to this, opinion is divided, most investigators agree that certain fats (especially butter) do have some effect on coagulation in that they shorten the Russell viper venom time. *In vivo* studies correlating changes in the clotting mechanism with those of the arterial wall may be of considerable interest[32] because the normal arterial wall produces enzymes which affect clotting, as in the clearing factor[33]. In the arteriosclerotic artery, the surface is more irregular, the lipid content is higher[34], the lipid breakdown products are different[35], and the atheromata themselves have a higher thromboplastic activity[36]. One should also keep in mind that the lipid composition of all organs is influenced by dietary fat, and this may in turn influence coagulation. In any event, the relation of fats to coagulation is still a wide-open field.

More recent work relating infectious processes to intra-arterial clotting may shed some light on arteriosclerosis. It is an old idea that infectious processes, by damaging the arterial wall, may be responsible for some of the changes seen in arteriosclerosis[37]. McKay has been able to produce intra-arterial platelet and fibrin deposits in pregnant rabbits by injecting endotoxins[38]. It is important that this process was observed when the organism was under the stress of pregnancy or when an endotoxin was injected together with substances of higher molecular weight[39]. It remains to be seen in which way these changes may be linked to arteriosclerosis.

The factors so far enumerated can modify the arteriosclerotic lesion, but are unspecific because they cannot account for the early occurrence of what is now believed to be the primary lesion in infants; neither can they account for the focal distribution of the lesions in the arterial system. These aspects of the disease invite the speculation that it may have a specific chronic infectious origin, as do leprosy, tuberculosis, and syphilis. Such a hypothesis could account for the early onset of the disease, for the focal distribution of the lesions, for the specificity of the organ attacked, for the eventual emergence of a histologically rather characteristic lesion from its early form, and even for the occasional disappearance of the lesion[40]. Furthermore, in view of the fact that the total population is affected by the disease at an early age, differences in the development of the lesions among members of the population would be accounted for by general factors such as heredity, nutrition, and the like. Something akin to this has actually happened in parts of the world where, at one time, syphilis infected the whole population and where, before the infectious origin of the disease was known, the clinical manifestations were ascribed to factors similar to those implicated in arteriosclerosis. Even the rapid increase in the consequences of the disease in younger age-groups may be explained by the known increase of an unchecked infection

if others are suppressed, as has happened in our lifetime. Finally, such chronic infectious diseases as syphilis and tuberculosis have been with the human race for a long time, and their natural history was characterized by sudden epidemics. Is it possible that we are witnessing such an outbreak of an old infectious disease?

Whether or not arteriosclerosis, as it is defined to-day, will remain a nosological entity or will be seen as several diseases, or whether it will be recognized as an unspecific response (like fever) to a number of etiological factors, is uncertain. Historically, the instrument of the physician of the nineteenth century was the microscope and of the twentieth century the paraphernalia of the biochemical laboratory. Notions about arteriosclerosis were formed accordingly. At the present time, virology and electron microscopy may enter the picture; it would be interesting to apply to these problems the methods used for the study of infectious nucleic acids[41]. Again, our sense of the etiology may be influenced by the methods we use.

Arteriosclerosis, being a part of the life process, can scarcely be pinned down to one etiological factor. Any investigator will, according to his temperament and talent, pick out just those of the infinity of factors which he believes are important; and one would be satisfied to elucidate one facet which would permit us to influence decisively the course of the disease.

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MOVEMENT IN THE ATMOSPHERE

THE Royal Meteorological Society held its summer meeting this year in the University of Exeter during July 12–14; this included two symposia, on "Winds and Circulation in the Upper Atmosphere" and "Medium Range Diffusion in the Atmosphere".

Winds and Circulation in the Upper Atmosphere

At this symposium the upper atmosphere was regarded as extending from the tropopause (10–15 km.) to the ionosphere (100 km.). This region is one of great interest to meteorologists since it is situated between the outer layers of the atmosphere, which are under direct solar control, and the troposphere, where the weather occurs. Due to indirect measurements and the more recent rocket investigations its general composition, temperature and wind structure are now fairly well known. Broadly, the composition is not very different from that in the troposphere: the temperature rises from 200–230° A. near the tropopause to 260–300° A. around 50 km., falls to 160–230° A. near 80 km. and then rises again at higher levels; the winds are mainly strong westerly in the winter hemisphere and easterly in the summer hemisphere. Many measurements have been made of the distribution of ozone, water vapour and atomic debris in the lower stratosphere and these have been used to infer the meridional circulations. Since electromagnetic forces, dissociation, excitation and ionization are still comparatively unimportant and mean free paths small, it is possible to apply the well-known meteorological equations used for the troposphere in the examination of its dynamics and interpretation of experimental observations.

The first paper, "Possible Meridional Circulations in the Stratosphere and Mesosphere", by R. J. Murgatroyd and F. Singleton, described a calculation of the meridional circulation sufficient to transport heat between the sources and sinks of radiation in a simple model of this region. The sources and sinks have been estimated from differences of solar energy absorbed by ozone and molecular oxygen and that emitted by long-wave radiation from carbon dioxide, water vapour and ozone. The principal sources are near the equatorial tropopause and in the mesosphere over the summer pole. The corresponding sinks are near the polar tropopause and the winter pole in the mesosphere. The calculation showed the air to be rising near, and possibly through, the equatorial tropopause, spreading polewards and sinking towards the polar tropopause. In the meso-

sphere the air rises near the summer pole, flows across the equator and subsides near the winter pole. This scheme is in good general agreement with many of the observed phenomena in this region, such as noctilucent clouds at high latitudes in the summer hemisphere near 80 km., comparatively high temperatures in the winter polar mesosphere, the 'sudden warming' phenomena, and the observed distributions of ozone, water vapour and atomic debris in the lower stratosphere.

The second paper, "Winds at 80–100 km. as revealed by Radar Reflexions from Meteor Trails", by J. S. Greenhow and E. L. Neufeld, contained the results of systematic work carried out at Jodrell Bank during 1953–58. The observation of drifts of ionized meteor trails using high-powered radio-echo equipment has produced upper wind data of a volume and accuracy unobtainable at other levels. Regular prevailing winds with westerly components of about 20 m.sec.$^{-1}$ have been observed each year in summer and winter with easterly components in spring and autumn. Large northerly components occur in summer and smaller southerly components in winter. There is a semi-diurnal periodic wind of about 20 m.sec.$^{-1}$ which can usually be represented by a vector which rotates in a clockwise direction with constant amplitude every 24 hr. Although large departures occur from this simple model, it provides good general confirmation of the resonance theory. Measurements of the 24-hr. and 8-hr. periodic wind systems and also non-periodic (turbulent) components were also described and comparisons made with Australian data. It is clear that with world-wide measurements of this type it will soon be possible to obtain a comprehensive picture of the atmospheric circulation at these levels. The final paper, "Fluctuations in Tropical Stratospheric Winds", by R. A. Ebdon, described detailed analyses of winds and temperatures found by radio-sonde and radar wind methods between about 15 and 25 km. near the equator. The winds were shown to have had a 26-months periodicity in east–west direction during the past ten years. The north–south components are usually small. This effect appears to be world-wide, greater at the higher levels and near the equator but observable up to 1,000–1,500 miles from it. When a change-over occurs, it starts at high levels and spreads downwards. At present there is no explanation for this phenomenon and there are dynamical difficulties in explaining how west winds can occur at all extensively in equatorial regions.

These three papers, which are being published in the *Quarterly Journal of the Royal Meteorological Society*, provoked considerable discussion. Recently, there has been increasing evidence that the humidity mixing ratio in the stratosphere may increase from about 2×10^{-6} to 4×10^{-5} between 15 and 30 km., and this is difficult to explain unless there is a large extra-terrestrial source of water vapour, but this seems unlikely. Recent measurements by the Meteorological Research Flight have shown values at 15 km. around 2×10^{-6} at all latitudes (although no measurements have yet been made at high latitudes in winter), so that vertical upwards moisture transfer, except perhaps by some penetrative convection, is ruled out for most latitudes. Other points needing clarification include the nature of the meridional transfer mechanisms, that is, the respective roles of the mean and eddy components on all scales. The next steps in the advance of our knowledge of the dynamics of this region will be to take account of the angular momentum as well as the energy balance, and to formulate criteria for the onset of dynamic instability (which depends on lapse-rates and wind shears and will therefore be different at different levels). Turbulent transfer will be more important in the lower stratosphere and the region between 50 and 80 km. than in the stable region between about 25 and 50 km., but the circulation here will of course also have to fit in with that for the region above and below it.

Medium-scale Diffusion in the Atmosphere

The second symposium was concerned with the propagation and spread of particles in the atmosphere up to distances of 100 miles or more from the source. This subject is of very wide application (since it involves all forms of atmospheric pollution including dust, radioactive materials, condensation and freezing nuclei and spores), and the study of concentrations at different points down-wind in different meteorological situations and their possible prediction is of great importance. In the first paper, "Some Observed Properties of Medium-scale Diffusion in the Atmosphere", by F. Pasquill, a series of experiments were described in which zinc–cadmium sulphide, a fluorescent pigment, was used as a tracer to investigate the horizontal spread of particles both in the cross-wind and along-wind directions. The particles were discharged either from a maintained point source on the ground and sampled at different heights down-wind by an aircraft, or alternatively dispensed from an aircraft flying across the wind (which could be regarded as a continuous line source) and sampled down-wind by instruments carried on the cable of a captive balloon. In each case it was possible to count under a microscope by using ultra-violet light the number of particles caught in unit volume at the different locations, and to relate them in terms of bearings and spread of the plume of particles to measurements of the strength of the source and the winds and other meteorological parameters.

When diffusion occurs over considerable distances and height-ranges the systematic wind shear with height affects the cross-wind and along-wind dispersion of material spread out in depth, and this effect depends on the amount of vertical mixing (both mechanical and convective). The question arises as to whether at medium ranges the horizontal spread of the particles bears a consistent relation to the apparent horizontal field of turbulence, as it does in short-range diffusion, or whether there is a contribution which cannot be provided by this turbulence and is recognizably due to shear. The analysis of the observations indicated that, when vertical mixing is present, the tendency for a cloud of particles to be sheared is strikingly removed ; but when vertical mixing is inhibited, distortion of the cloud takes place, yielding horizontal displacements which would become effective on the revival of the mixing. Due to this effect there is usually a diurnal cycle of vertical but not horizontal spread.

The details of intermediate cases require further examination for a full quantitative understanding, although it is clear that turbulent spread, especially that due to the low-frequency components, always makes a large contribution apart from the shear. There was also usually only a small variation with height of the direction and speed of travel of the particles compared with that of the wind. In some cases, however, the mean direction was several degrees backed from the direction of the mean wind in

the layer, and even from that of the surface wind, and was difficult to account for in terms of vertical momentum transfer.

Dr. J. M. Hirst then presented a paper on "The Long-range Travel of Fungus Spores". Plant diseases such as potato blight and black rust of wheat can be transmitted hundreds of miles due to the diffusion of spores in the atmosphere, and an examination of this mechanism is of considerable economic importance. At the place of origin, the spore concentration may be as much as 1/c.c. and the concentration in the vertical is controlled by turbulence and atmospheric stability. The decrease at inversions is usually rapid. Furthermore, the presence of an immigrant as opposed to a locally produced spore can often be recognized by its different vertical distribution. The concentration of the former tends to increase with height over the first few thousand feet and then decrease above, an effect which appears to be due to dry deposition during its relatively long period of travel. The efficiency of raindrops as collectors of spores is high, so that spores are quickly removed from the atmosphere during rain. Examples were shown of the measurement of the spread of spores of black rust of wheat from Europe to Britain. The disease appears in early summer in southern Europe and slowly spreads northwards and is often observed in Britain by about August. Samples obtained over the English Channel using the aircraft of the Meteorological Research Flight during periods of southerly winds had produced vertical profiles of spore concentrations which illustrated their travel in the atmosphere from Europe, whereas on other occasions it had been demonstrated by back-tracking air trajectories that they were not present in air of Atlantic origin reaching samplers on the ground. Work of this type is continuing, and further experiments are now in hand to examine the diurnal variation of spore content at various heights and other characteristics of their diffusion in the atmosphere.

The final paper, "Mechanisms of Atmospheric Diffusion", by R. S. Scorer, was concerned with diffusion by turbulent eddies and by thermals. In certain meteorological situations the latter are very important and may even be the dominant mechanism. When diffusion of momentum occurs following any disturbance in the flow, there will be a loss of energy as the energy of shear is disorganized into smaller fluctuating motions, a process which may best be studied in terms of the vortex lines in the flow (Scorer, R. S., *Natural Aerodynamics*, Pergamon Press, London, 1958). Momentum, however, is not always transferred at the same rate as heat or material, and in the case of thermals, $\frac{K_{\text{momentum}}}{K_{\text{material}}} \doteq \frac{\sigma}{2}$, where σ is the fraction of the horizontal area occupied by the thermals and is usually 0·1–0·01. Hence thermals are about 20 times more efficient at transferring material than momentum, whereas in the case of mechanical stirring their rates of transfer are more equal. As a consequence, the wind shear can be retained, and there can still be considerable transfer of material when thermals are operating.

The ensuing discussion was principally concerned with the generation of thermals, which are intermittent and frequently confined to preferred areas, the relative importance of form drag and viscous drag and the necessity of considering eddies in three rather than in two dimensions. As regards the practical aspects, it now appears that, observationally and empirically, at least the problem of determining the concentration of particles down-wind from a given source in given meteorological conditions is well understood and considerable progress has now been made.

R. J. MURGATROYD

UNIVERSITY EDUCATION IN GHANA

By DR. C. T. MORTIMER
University College of North Staffordshire, Keele, Staffordshire

THE report of the Commission on University Education in Ghana, and a statement by the Government on the report*, were published on July 5, 1961. Mr. Kojo Botsio, a Minister of the Ghanaian Government, was the chairman of the Commission, which included American, British, Russian and Sierra-Leonean members. The original terms of reference were "to advise on the future development of university education in Ghana, in connection with the proposal to transform the University College of Ghana, at Legon, and the Kumasi College of Technology into an independent University of Ghana". Afterwards, these terms were altered slightly, because the Government later proposed that the two institutions should be independent universities. They are to be called the University of Ghana and the University of Science and Technology, Kumasi.

In formulating their constitutional proposals for these two Universities, the Commission was guided by two main principles. These are that the Universities should be able to respond to the immediate and future needs of the community, and that they should have the greatest possible autonomy in their organization, teaching and research. The report deals first with the relationship between the Universities and the Government of Ghana. The prevailing opinion was that new developments in higher education should be planned and financed at a national level by a single body, which would also co-ordinate and finance research programmes, both within and outside the Universities. It was recommended that a National Council for Higher Education and Research be established. Prof. L. A. Bornholdt, of the University of Pennsylvania, did not support this recommendation. "If such a body were to be created and were given full responsibility for planning research and for financing university education throughout Ghana, the Commission's recommendations for the autonomy and freedom of individual institutions would become no more than pious hopes." This minority opinion suggested a division of responsibilities and the creation of three bodies in place of the National Council: a Joint Research Board, a

* Report of the Commission on University Education. Pp. 43. 4*s*. Statement by the Government on the Report of the Commission on University Education. W.P. No. 5/61. Pp. 7. 6*d*. (Accra, Ghana: Ministry of Information; London: Crown Agents for Overseas Governments and Administration, 1961.)

University Grants Committee, and a Research Grants Committee. The Government has accepted the majority opinion and has created a National Council.

Although the Commission made no specific recommendation, the Government has appointed *interim* University Councils, which have taken over the responsibilities of the College Councils until the governing bodies of the new Universities have been established. The Government Statement notes that these *interim* Councils have been unable to offer appointments to five members of the existing academic staff. The *interim* vice-chancellors and the Secretary of the National Council will be asked to report on recommendations of the Commission and those of committees which have been appointed by the President of Ghana. Some of the functions of these committees were: (1) to prepare proposals for the constitution of the National Council ; (2) to draft constitutions for the new Universities ; (3) to consider the supply of students ; (4) to consult with College authorities on syllabuses.

The Commission considered in some detail the type of university government which would best meet the needs of higher education in Ghana. For the composition of the University Councils, the Commission suggested the following : the Chancellor, the Vice-Chancellor, and one member appointed by the Council of the sister University. Of the remaining members, one-third should be nominated by the Government, one-third by various educational bodies, and one-third should be selected by the University Senate from its membership. Membership of the Senate would be broadly representative: the head and two other teaching members of each Department, at least one of whom should be Ghanaian.

In its statement, the Government endorses the views of the Commission on the steps which should be taken to accelerate the Africanization of University staff, in order that there will be a substantial majority of Ghanaians by 1967–70. The report notes that, at present, of a total academic staff of 201 at Kumasi and Legon, only 35 are Ghanaians. Ghanaians who are of professorial calibre, working in departments which already have professors, should be appointed either as additional full professors or as associate professors. When professorships fall vacant in departments in which there is no Ghanaian immediately qualified for appointment, distinguished scholars from other parts of the world might be brought in as visiting professors for periods of two or three years, while the departmental responsibilities would be entrusted to a Ghanaian lecturer. The report stresses that efforts to increase the proportion of Ghanaians on the staffs of the two Universities should not involve the displacement of existing expatriate members of the staff. In fact, the Government recognizes that the expansion of higher education will make it necessary to continue, for some time to come, the recruitment of overseas personnel on short-term contracts.

The total 'high-level man-power' requirement during the period 1960–65 in administrative, managerial and professional employment and in secondary school teaching has been estimated at 7,000, or an average annual requirement of 1,400. To meet this there must be an increase, as rapidly as possible, in the flow of students into the two Universities. Under the present entrance regulations, possible student numbers are controlled by the number of those obtaining the General Certificate of Education with two subjects at advanced level. This consequently depends on the extent to which sixth-forms have been developed in the secondary schools. On the basis of existing sixth forms and plans for expansion of sixth-form work, it has been estimated that the student population of the Universities in 1964 would be about 1,400, representing an annual output of some 450 graduates. This estimate does not take into account courses at Kumasi in such subjects as engineering, architecture and pharmacy, which can be entered at the ordinary level of the General Certificate of Education. To ensure that the rate of development of university education is not so dependent on the rate at which sixth forms can be developed, it is proposed that there should be a special entry into the University of Ghana for students who have passed General Certificate of Education (ordinary level), but who lack the normal advanced-level qualifications, similar to the arrangement which already exists at Kumasi. By 1964 the estimated annual output of General Certificate of Education ordinary-level certificates will be about 1,800.

It has been agreed that the two Universities should remain predominantly residential, though making provision for a proportion of non-residential students. To accommodate the increased student numbers at Legon, the Government has authorized the building of a fifth hall of residence, to accommodate 320 students. At the same time plans are to be drawn up for the building of hostel-type annexes to the four existing Halls, which at present can accommodate about 700 students. It has often been suggested that the system of hall membership has tended to inhibit the growth of student life and restrict students' social activities. To overcome these objections, and to cater for non-residential and part-time students, the Commission has proposed that centrally situated cafeterias, common rooms, gymnasia and other common facilities for staff and students should be built at both Universities.

With the ending of the 'special relationship' with the University of London, the time was considered opportune to review the structure of degree courses. Many of the suggestions presented to the Commission on this matter would be met by an arrangement whereby all students in their first year would take one of a limited number of prescribed general courses. Each of these courses would normally include three subjects. Students could then proceed either to a two-year general degree course in two or three subjects or to a special degree course in one or two subjects, terminated by a final examination. In addition, all students at both Universities would take some courses in African studies. Since much of the reorganization of the structure of degree courses has already been under consideration, it is hoped that students entering the University of Ghana in October 1961 would begin at once to work for the B.A. and B.Sc. degrees of the new University.

In considering the division of responsibilities for teaching and research between the existing Colleges at Legon and Kumasi there were three areas in which actual or potential overlapping existed—in engineering, agriculture, and in work for general degrees. The Commission recommended that engineering be concentrated at Kumasi, together with architecture, building technology, rural and urban planning, and pharmacy. The four-year diploma course in agriculture at Kumasi should be strengthened, although the degree course in agriculture should be confined to Legon. It is hoped that with the development of science laboratories at Kumasi, it might be possible

to start general degree courses in some combination of science and technology ; metallurgy, wood technology, agricultural engineering and industrial chemistry are cited as examples. The Government accepts the Commission's proposal for the establishment of an Institute of African Studies, based on the University of Ghana, but having some measure of autonomy. The future of the College of Business Management at Achimota remains uncertain, and the National Council will examine ways of associating the College and also the Ghana School of Law with the Universities. The Commission welcomed the suggestion made by Prof. Carl Iversen, vice-chancellor of the University of Copenhagen, that the Government of Denmark might be willing to associate itself with a scheme for establishing further residential adult colleges in the various regions of Ghana.

In addition to the main proposal that the Colleges at Legon and Kumasi should become independent Universities, the Commission has recommended that a University College of Cape Coast be established. This College would be in some relationship with the University of Ghana, though few of the details have been worked out.

As one phase of the development of higher education in Ghana ends and another begins, the Government has paid tribute to the encouragement and practical assistance given to the Colleges by the University of London, the Inter-University Council for Higher Education Overseas and the Council for Overseas Colleges of Arts, Science and Technology. It records with satisfaction the high standards already achieved by the Colleges and its confidence in their future as independent Universities.

OBITUARIES

Dr. Otto Maass, C.B.E., F.R.S.

Dr. Otto Maass, one of the most eminent of Canadian chemists, died in Montreal on July 3 at the age of seventy. As assistant to the President of the National Research Council during the war years, director of chemical warfare in the Department of National Defence and later as scientific adviser to the Chief of General Staff, he was well known to a multitude of colleagues in both the United Kingdom and the United States. As general director of the Pulp and Paper Research Institute of Canada, he had a wide acquaintance in the pulp and paper industry. But he is best and most affectionately remembered by the many generations of graduate students who gained their first insight into the significance of physical chemistry and the meaning of chemical research under his stimulating guidance.

Otto Maass was born in New York City on July 8, 1890, but went to Montreal with his parents at an early age. He was educated in Montreal High School and McGill University, obtaining the B.A. degree in 1911 and the M.Sc. in 1912. He was awarded an 1851 Exhibition scholarship and went to Berlin in 1913 to work in the laboratory of Prof. Walther Nernst. Trapped in Germany by the outbreak of war in the following year, he was able to escape across the border into Switzerland and thence return home. His interrupted studies were continued at Harvard under the late Prof. T. W. Richards. He submitted a thesis and received the Ph.D. degree from Harvard in 1919.

In 1917 he was appointed lecturer in physical chemistry at McGill. In 1923 he became Macdonald professor of physical chemistry and, in 1937, chairman of the Department, appointments which he occupied until his retirement in 1955.

From the very outset of his teaching career, he began to be surrounded by a rapidly expanding group of enthusiastic young graduate students eager to share in the development of his ideas. One very early achievement was the preparation, for the first time, of pure hydrogen peroxide ; and this gave rise to a whole series of publications reporting accurate measurements of its physical properties. Another early investigation involved devising a simple and accurate method of measuring densities of condensable gases. This was ultimately refined to the point where reliable figures for the atomic weights of carbon and sulphur were obtained from the molecular weights of carbon dioxide and sulphur dioxide. Meanwhile, similar techniques had been applied to observe density changes on mixing two gaseous components and to detect reaction between two components in the vapour phase. This led on to a comparison of reaction velocities below and above the critical temperature which, in turn, gave rise to the prolonged series of investigations of critical phenomena which brought about a wholesale revision of ideas concerning the critical region.

As early as 1920, Maass became aware that very few reliable physical data were available on sulphur dioxide solutions such as were used in the manufacture of sulphite pulp. Work in this field was followed by a series of investigations of the factors influencing the rate of penetration of sulphite solutions into wood chips. In 1928 the Pulp and Paper Research Institute of Canada was founded, and by arrangement with the Graduate Faculty of McGill, Maass and his students were enabled to pursue further researches in the laboratories of the Institute. He became its general director in 1940 and was instrumental in initiating a variety of investigations of problems directly concerned with the war effort.

On his retirement in 1955, Maass was appointed a principal research officer in the Division of Chemistry, National Research Council, and he spent the next three years in the laboratories of the Council in Ottawa. Then he returned once more to McGill as a research associate in the Chemistry Department. Much of his time was spent in his laboratory in the Chemistry Building up to the day of his death.

Space does not permit even a bare enumeration of the degrees, medals, memberships of learned societies and other honours which came to him. It would seem, in any event, superfluous to those who knew him. His work was his life and brought with it its own reward. By his work he will be remembered.

J. H. Mennie

Prof. R. F. Naylor

Ralph Francis Naylor, professor of chemistry in the Royal College, Nairobi, was killed in a motor accident at Mkushi in Northern Rhodesia on August 6. In this tragic accident, at the regrettably early age of thirty-nine, one who has done much for

chemistry and for university education generally in East Africa was lost.

Born in 1921 in London, Ralph Naylor was educated at Emanuel School and at the Imperial College of Science and Technology, London, where he graduated B.Sc. with honours in 1942. He then joined the British Rubber Products Research Association where he worked on olefinic reactivity and aliphatic sulphur compounds. Some twelve publications on this work resulted, including one in *Nature*, over the period 1943–1949.

In 1949 he went to Makerere College as a lecturer in organic chemistry and became a senior lecturer three years later. In September 1953 he was given nine months leave of absence to work with Dr. J. H. Hanks in the Department of Bacteriology and Immunology at Harvard, in which he was assisted by Smith-Mundt Research and Fulbright Travel Grants. He returned to Makerere as acting head of the Chemistry Department. The head of Department, Prof. Tenniswood, had died shortly before, also in a motor accident. He acted in this capacity for nearly two years. Although one of the new chemistry buildings had just been completed, much of the design of the second building and the planning for future expansion, which he performed enthusiastically and in masterly fashion, fell to him.

In spite of these increased responsibilities, Naylor continued his research on the chemotherapy of leprosy drugs. He took much interest in leper colonies and villages throughout Uganda and neighbouring territories. He was on the Board of Governors of the Kumi Leprosy Settlement, which he visited regularly. Having decided that a radiochemical approach to this problem was likely to be fruitful he spent most of one long vacation at Harwell learning tracer techniques, so that he might design and equip a radiochemical laboratory in the Chemistry Department at Makerere. He read a paper on his work at the seventh International Congress on Leprosy in Tokyo in 1958.

The East Africa Section of the Royal Institute of Chemistry held its first annual general meeting early in 1956. Ralph Naylor was one of those responsible for its foundation. He became the Section's first secretary, and up to the time of his death he never relaxed in arranging, organizing and reporting meetings, visits, excursions and exhibitions. East African chemists owe him a particular debt in this connexion.

At Makerere he always took an active personal interest in students and student affairs, particularly in his own tutorial group. He was for a time a resident tutor, and later deputy warden of Mitchell Hall, one of the student halls of residence. He was much sought after as a lucid lecturer, an enthusiastic committee member and an impartial chairman. Ralph Naylor was a man of deep religious conviction. He was the founder leader of a Boys' Crusader Bible Class in Kampala, and also for some years secretary of the Uganda auxiliary of the British and Foreign Bible Society.

His interest in open-air activities was shown by his love of travel and mountain climbing. He was for a time president of the Uganda Mountain Club, and had made ascents of several of the difficult Ruwenzori peaks as well as other mountains in East Africa. He was also a trustee of the East Africa Outward Bound organization, which has its headquarters at Loitokitok at the base of Kilimanjaro, and carries out its activities on that mountain. He had also been president of the Uganda Amateur Athletic Association at the time when Uganda was beginning to play a part in international athletics. Photography was one of his hobbies.

In 1955 he married Miss Dilys Rees. She was slightly injured in the accident through which he died, and their son Paul, aged four, rather more seriously.

In January 1961 he took up duty as first professor of chemistry at the Royal College, Nairobi, and in his short time there he was also dean of the Faculty of Science, and worked enthusiastically on the planning of the new chemistry building. It is sad that he will not now see the building through to final completion and occupation.

It will thus be clear that Ralph Naylor was characterized by the energy which he put into so many diverse activities, and the thoroughness with which he carried them out. He took a pride in meticulous planning down to the finest detail and his plans nearly always succeeded. His loss will be felt by his many friends and by all who have an interest in scientific education and development in East Africa.

MALCOLM CRAWFORD

Sir Arthur Olver, C.B., C.M.G.

WITH the death on August 15 of Sir Arthur Olver at the age of eighty-six, the veterinary profession has lost one of its most eminent authorities on livestock development. The son of a successful Cornish livestock breeder, he was early introduced to the exactions of animal husbandry. After studying in London and becoming a member of the Royal College of Veterinary Surgeons, of which he later became a Fellow, he was commissioned in the Army Veterinary Department some months before the outbreak of the South African War.

He saw active service with the cavalry, and later, as senior veterinary officer, Natal, first showed his flair for administration.

In 1904 he obtained permission to work under Theiler at his Daspoort laboratory when that authority was so successfully competing with the prevalent epizootics, as well as demonstrating with exactness connexions between nutrition and disease. In 1906, while still a captain, he was appointed principal veterinary officer, Egyptian Army and the Sudan Civil Service. In his first year he stamped out rinderpest in the Nile Valley, organized the Sudan Veterinary Service and enabled the cattle trade between Central Africa, the Sudan and Egypt to be reorganized on a satisfactory basis, for which he was awarded the Order of Osmanieh, 4th class.

He then was appointed assistant director general, Army Veterinary Service, at the War Office. For the greater part of the First World War he served at headquarters of the British Expeditionary Force and besides being mentioned in dispatches four times was made a C.M.G. In 1917 he was sent to re-organize the veterinary arrangements of the Remount Commission in the United States and Canada, where equine infectious diseases were causing havoc. Once again his efforts were successful and he was made a C.B.

In 1930, when a colonel, he retired from the Army to become animal husbandry commissioner with the Government of India, an appointment which had been created on the recommendation of the Royal Commission to investigate the state of agriculture in India. During his eight years in that office he brought under centralized supervision all aspects of official

livestock research and development throughout the subcontinent, drafted an all-India Act for the Control of Contagious Diseases of Animals, defined and published a description of most of the important breeds of cattle in India and organized the All-India Cattle Show Society as well as originating research and promoting veterinary education. He was knighted in 1937.

He left India in 1938 to become principal of the Royal (Dick) Veterinary College in Edinburgh, which he administered through the difficult war years that followed. He retired from Edinburgh and public life in 1946 to farm with the enthusiasm and zest with which he had pursued all his undertakings.

He had many outside interests, but he excelled at, and derived most pleasure from, field sports. Since an illness some five years ago lessened his physical activity he took up painting with the single-minded concentration so characteristic of him. Two of his paintings were accepted by the Royal Academy, and he was greatly amused that a critic predicted a promising career for the artist.

He is survived by a widow and one son.

G. WILLIAMSON

NEWS and VIEWS

The Royal Radar Establishment: Mr. W. J. Richards, C.B., C.B.E.

MR. W. J. RICHARDS resigned from the post of director of the Royal Radar Establishment in July in order to become first director of the new Staff College for Further Education recently established by the Ministry of Education (*Nature*, **190**, 308; 1961). He joined the Scientific Civil Service in 1925, and was attached to the Royal Aircraft Establishment, Farnborough, where most of his active research work was carried out, his main interest being in the development of instruments for use in aircraft. He was appointed head of the Instrument Department in 1936. After a period at Ministry of Aircraft Production Headquarters in London, Mr. Richards succeeded Dr. W. B. Lewis in 1946 as chief superintendent of the Telecommunications Research Establishment, Malvern, and when this was amalgamated with the Radar Research and Development Establishment in 1953 (*Nature*, **172**, 277; 1953) he was made director of the combined establishment now known as the Royal Radar Establishment. Under his directorship the amalgamation was achieved in a happy and efficient manner and the Royal Radar Establishment became a central research establishment for electronics serving both the Royal Air Force and the Army. It has played a notable part in the expansion of Britain's defence system, and, in particular, has made significant contributions to the electronic aspects of guided weapons, to the electronic equipment of the V-bomber force and to the modernized early-warning radar. The Establishment has achieved an international reputation for fundamental physics research, particularly in the physics of solids, and has pioneered in the development of many new radio techniques now commonly used in industry. One of Mr. Richards's main interests has been in education, and training of new staff members. It was largely due to his initiative that the Malvern College of Electronics was founded, in association with the Royal Radar Establishment. His new post will give him further scope for the development of these interests.

Dr. G. G. Macfarlane

DR. MACFARLANE, at present deputy director of the National Physical Laboratory, has been appointed to succeed Mr. Richards as director of the Royal Radar Establishment. Dr. Macfarlane, who is forty-five, graduated with first-class honours in electrical engineering at the University of Glasgow in 1937, and went on to do two years postgraduate research at Dresden, where he obtained the degree of Dr. Ing. in 1939. He joined the Telecommunications Research Establishment (now the Royal Radar Establishment) in 1939. Throughout the War years he concentrated on mathematical problems in radar and microwave physics. In 1945 he became head of the Mathematical Group and a year later took charge of the Theoretical Physics Division. In 1953, Dr. Macfarlane began individual research work on semiconductors in the Physics Department at the Royal Radar Establishment. In a series of fundamental studies of the optical properties of germanium and silicon he and his group obtained and explained detailed fine structure in the absorption spectra of germanium and silicon over a wide temperature-range. They demonstrated the occurrence of excitons and phonon-assisted indirect transitions in the absorption edge spectra of these semiconductors. He continued this work until early 1960 when he became deputy director at the National Physical Laboratory with special responsibilities for relations between the Laboratory and industry. Dr. Macfarlane will take up his new appointment at Malvern in March 1962.

Chemistry in the University College of Sussex: Prof. C. Eaborn

DR. C. EABORN has been appointed professor of chemistry in the new University College of Sussex. He is best known for his publications on the chemistry of organosilicon compounds, including a book of which Prof. H. J. Emeléus recently wrote, "The outstanding impression on reading this book is that, for the first time, order has been introduced into the widely scattered literature". His major research contributions have involved mechanistic studies of reactions of silicon–hydrogen and silicon–carbon bonds, particularly cleavages of aryl–silicon bonds. He has shown that these cleavages are usually electrophilic aromatic substitutions, and with his co-workers has accumulated a large body of information on effects of substituents on the ease of such substitutions, has extended the study to include the reactions of aryl-germanium, -tin, and -lead bonds, and he has shown that these cleavages are useful in synthesis; for example, they can be used to attach a nitro- or sulpho-group at a specified position of an aromatic compound. Recently, Dr. Eaborn and his co-workers, using a simple method they devised for counting tritium, have carried out detailed studies of the rates of acid-catalysed aromatic hydrogen-exchange reactions, and the results, combined with those from the aryl–metal cleavages mentioned here, have been

used to test and modify concepts of electrophilic substitution based in the past on more familiar reactions such as nitration. Incidental observations in the course of Dr. Eaborn's work which attracted considerable attention were the first isolation of an optically active organosilicon compound having a silicon as its single asymmetric centre, and his detection of the mesomorphism of di-isobutylsilane diol, which does not fall into any one of the three classes previously thought adequate for classification of liquid crystals.

Dr. Eaborn's researches involve at present, in addition to extensions of the work mentioned here, a general study of mechanisms of reactions of organometallic compounds, including the effects of high-energy radiation on such compounds, and investigation of the reactivity of fluorene derivatives. Dr. Eaborn is a graduate of the University of Wales, of which he holds the D.Sc. degree. He was appointed an assistant lecturer in the (then) University College of Leicester in 1947, promoted lecturer in 1950, and in 1954 was given the title of reader in physical-organic chemistry in recognition of his distinguished work in that field. He has recently been awarded a Robert A. Welch visiting scholarship at the Rice University in Texas, which he expects to take up early in 1962.

The Falkland Islands Dependencies Survey

THE R.R.S. *Shackleton* left Southampton on October 3 for the Falkland Islands Dependencies Survey, 1961–62, taking twenty scientists and technicians of the Survey and two meteorologists for South Georgia. The R.R.S. *Shackleton* has been extensively used since it was taken over by the Survey in 1955. Earlier this year, using the *Shackleton*, the Survey was able to collect geological specimens from the Clerke Rocks and to make landings on Visokoi Island (South Sandwich Group), Leskov Island and Vindication Island. During the same voyage, a magnetic survey of the Scotia Arc (the submarine mountain range running from South Georgia through the South Sandwich Group and the South Orkneys to the South Shetlands) was carried out. Mr. Cameron and Mr. Kennett, who were responsible for this survey, hope to take seaborne seismic soundings this season in the same area, extending their magnetic study of the rocks below the ocean floor, with the view of tracing the course of the Arc and its connexion with the Andean mountains of South America. Also arranged for the R.R.S. *Shackleton* is a series of regular observations to check the secular change in the Earth's magnetic field; this investigation is being made at the request of Prof. Takesi Nagata, Tokyo University, on behalf of the International Union of Geodesy and Geophysics.

The purpose of the Falkland Islands Dependencies Survey, the director of which is Sir Vivian Fuchs, is to carry out scientific research in the Antarctic, under the administrative control of the Governor of the Falkland Islands. For nearly seventeen years it has maintained permanent bases on the mainland of Graham Land and on nearby islands. At these bases between ninety and one hundred scientists and technicians spend periods of up to two years carrying out a systematic series of investigations which, so far, have been producing valuable information about the weather and structure of the south polar continent. In addition, numerous investigations are being made in the fields of geophysics and human physiology in Antarctic conditions. Each Antarctic summer (November–March) the sea ice clears sufficiently for specially strengthened ships, such as the R.R.S. *Shackleton*, to make voyages to these bases with relief staff and stores.

Russian Space Research

RECENT cosmic flights in the U.S.S.R. found their reflexion in the popular scientific journal, *Priroda*, for 1961. Thus, N. M. Sisakyan (**1**, 7) discusses the biological factors, while space–time problems are discussed by D. A. Frank-Kamenetsky (**1**, 16) and by A. S. Kompaneets (**5**, 17); V. G. Kurt (**2**, 23) discusses the upper atmosphere and the interplanetary space. V. D. Davydov (**3**, 3) concerns himself with the Earth–Venus flight, and finally in No. 5 (May) of *Priroda* there is a number of articles dealing with the flight of Y. A. Gagarin on April 12 (pp. 3, 5, 6, 8, 14, 16). The space ship which circumnavigated the Moon on October 7, 1959, automatically photographed the other side of the Moon, and an account of these photographs and of the map constructed of the other side of the Moon are given by Y. N. Lipsky (*Priroda*, **1**, 35; 1961); a map on the scale of 1 : 10,000,000 is appended.

Atomic Energy and its Applications

Atoomenergie en haar Toepassingen (Atomic Energy and its Applications), the scientific and technical periodical published under the auspices of The Netherlands Reactor Centre (Reactor Centrum Nederland), normally contains articles in Dutch dealing with various aspects of nuclear energy, but the June issue (**3**, No. 6), which is devoted entirely to the activities and achievements of the nuclear industry in The Netherlands, is printed in English for the benefit of a wider public. The introductory article, which is written by Prof. M. Bogaardt, adviser to the Centre, gives a general picture of the industrial activity in the nuclear field in The Netherlands, and is followed by a brief illustrated description of the Centre and a number of short articles contributed by leading Dutch firms. The comprehensive buyers' guide of nuclear and allied products, materials and services—the first of its kind—which concludes the issue, has been compiled from answers received to a questionnaire circulated among a large number of Dutch firms which are actively engaged in the nuclear field. The guide consists of two sections, the first being an alphabetically arranged classified list of products with the names of the respective manufacturers, and the second an alphabetical index of firms and their addresses with brief details of their chief products.

Society for Folk-Life Studies

A VERY successful conference to inaugurate the Society for Folk-Life Studies was held at University College, London, during September 6–8, 1961. More than eighty people from many parts of Great Britain and Ireland attended and, at a general meeting held on September 8, the Society was formally established. The following officers were elected: *President*, Dr. Iorwerth C. Peate (Welsh Folk Museum); *Vice-President*, Mr. Andrew Jewell (University of Reading); *Secretary*, Mr. J. Geraint Jenkins (Welsh Folk Museum); *Treasurer*, Mr. Stewart F. Sanderson (University of Leeds). The following members were elected to serve on the Council: Mr. Frank Atkinson (Barnard Castle, Durham); Mr. G. Ewart Evans (Ipswich); Mr. A. T. Lucas (National Museum of Ireland); Mr. B. R. S. Megaw (University of Edin-

burgh); Mr. C. S. Mundy (University of London); Dr. Melville Richards (University of Liverpool); Mr. G. B. Thompson (Ulster Folk Museum); Mr. D. M. Wilson (British Museum).

Membership is open to all individuals and institutions interested in the study of the traditional patterns of life both in rural and urban communities. Although various bodies such as the Folklore Society, the British Agricultural History Society and several other societies are each interested in particular aspects of folk-life and social history, this new Society to cover the complete field fulfils a long-felt need. It can serve as a focus for the activities of those individuals, amateur and professional alike, who take an interest in their native culture. In addition to holding an annual conference at various centres in Great Britain and Ireland in turn, the Society will also publish its own journal. Further information can be obtained from the Secretary, c/o Welsh Folk Museum, St. Fagans, Cardiff.

The Mankind Quarterly

A recently published new journal seems to be devoted to the promotion of the idea of the basic significance of the biological endowment of human races in determining psychological and cultural characteristics (*The Mankind Quarterly: An International Journal dealing with Race and Inheritance in the Fields of Ethnology. Ethno- and Human Genetics. Ethno-Psychology. Racial History. Demography and Anthropo-Geography.* Vol. 1, No. 4, April 1961; Vol. 2, No. 1, July–September 1961. Edinburgh. 6*s.* each). It presents some descriptive articles, for example, on the Kurumbas and Todas of the Nilgiri Hills of South India (by Ruggles Gates) and on Bantu facial conformation (by J. D. J. Hofmeyr and H. Hitzeroth), and some cautiously analytical ones, for example, on "the cultural hypothesis" of mental types (by Gutorm Gjessing). But most of the contributions argue strongly that there is a close relation between innate physical and mental racial characteristics. In various contexts it is stated: that there is a genetic predilection for certain cultural forms; that there is a significant correlation between the occurrence in ancient times of "the Aryan speech" and the peoples of largely Nordic strain: that within any society an intellectually *élite* group tends to maintain its genetic superiority through selective mating. The "equalitarian dogma" of Negro aptitudes—as illustrated by Gunnar Myrdal's work—is attacked, and it is implied that social segregation of the Negro in the United States would be a proper course. The material in this journal is of very uneven quality and it is unreliable as a guide for students.

The South Australian Museum

Museums in many places are, at the present time, undertaking extensive re-organization schemes. The report of the Board of the South Australian Museum for 1959–60 is yet another example of this world-wide movement, and it is only labour and finance which dictate the speed of these transformations (Pp. 16. Adelaide: Government Printer, 1961). The activities of the Museum include television broadcasts, a display of Australian wild flowers at the Adelaide Festival of Arts and an extensive programme of field work both in archæology and natural history. A rather unusual feature is the formation of an underwater research group, the members of which have collected marine invertebrates and have been especially successful in stupefying fish by means of the distribution of rotenone in the wreckage of a ship lying in 50 ft. of water. The fish are collected in nets by the aqualung divers. Members were fortunate in being able to observe and capture two specimens of the rare leafy sea-dragon, *Phycodurus eques*.

Neurology of Language

In a wide-ranging review of the neurology of language, Sir Russell Brain shows how difficult it is to infer from cases of aphasia the physiological organizations underlying speech (*Brain*, 84, Part 2; 1961). He also shows the inadequacy of purely psychological classifications of aphasia and of the attempt to correlate disturbances which can be classified psychologically with lesions in particular situations. The physiological organizations on which speech depends are of great complexity, extending over considerable areas of the brain, and are organized in time as well as in space. These serve psychological functions, but the breakdown of a physiological schema does not necessarily, or indeed usually, disturb speech in a way that corresponds to a single type of psychological defect. Moreover, the anatomical organization of the schemas means that only rarely will a single type of schema be disturbed in isolation from others. What the clinician calls aphasia is almost always the product of a mixture of physiological disturbances resulting in a complex medley of psychological disabilities. For the present, the classification of the aphasias must be largely empirical. The task for the future is to use all the available modern methods of psychological testing, linguistics, phonetics and communication theory, to correlate psychological with physiological functions. Already the directions in which progress is likely to be made can be seen. From a number of investigations it is hoped to arrive at an analysis of speech disturbances at both the psychological and physiological levels, and of their interrelationships, which will provide a scientific basis for the recognition of the type of disorder of function present in each individual aphasic patient.

Immunization against Disease

The subject for discussion at the Thirteenth World Health Assembly in 1960 was "The Role of Immunization in Communicable Disease Control". A recently published volume contains the introductory address of the chairman, Prof. Zhdanov of Moscow; papers on the background of immunization, by Prof. Cruickshank of Edinburgh, and the efficiency of immunization procedures used in public health practice by Prof. Edsall of Harvard; there is also a paper on compulsory or voluntary vaccination, by Dr. J. de Moerloose of the World Health Organization, and finally, the report of the technical discussion (*The Role of Immunization in Communicable Disease Control*. Various authors. World Health Organization: Public Health Papers. No. 8, 1961. Pp. 118. Price 6*s.* 8*d.* Also published in French. Available through H.M.S.O.). There are nowadays so many vaccines and immunization schedules that the average practitioner is understandably bewildered and uncertain which to use and when. This small volume contains authoritative and up-to-date views on the value of the various vaccines, and the pros and cons of immunization as well as the most suitable times for vaccination.

Producing Transplantable Human Tissues in the Laboratory

THE July issue of *Discovery* contains an article by Dr. D. Petrucci, Medical Biological Studios, Bologna, entitled "Producing Transplantable Human Tissue in the Laboratory". The article resulted from Dr. Petrucci's original work on building tissues which, in contrast to adult tissues, would be transplantable and acceptable by adult organisms. The idea was that the chances of a glandular transplant surviving would be improved if the gland were obtained from an embryo that is first cultivated outside the maternal body. The technique was to fertilize the ovum between two chamois disks, in what Dr. Petrucci calls his "biological cradle". Various 'cradles' have been used in which the developing zygote and embryo could be photographed continuously. The article is extensively illustrated, and the 48 illustrations show the fertilization and development of the embryo covering a period of 58 days of growth from fertilization; they also demonstrate the 'cradle'. In a field so sparse in workers, who themselves tend to be highly sceptical of all and everybody's work in that field, it is a pleasant relief to find a general article. The illustrations will be of great use to science teachers.

Geology and Ceramics

To mark the occasion of the Seventh International Ceramic Congress, held in London during May 1960, a special exhibition of rocks and minerals used by the British ceramic industry was shown at the Geological Museum in South Kensington. A booklet (*Geology and Ceramics*. Pp. ii+28. London: H.M.S.O., 1961. 3*s*. 6*d*. net), prepared by Dr. P. J. Adams, was issued to give a brief account of the rocks and minerals used in the industry, describing their nature and geological occurrence. The many ways in which the raw materials are used by the ceramist, potter and engineer are also described. The booklet is well illustrated and the subject-matter is dealt with under the headings of pottery and whitewars, structural clay products and refractories and technical ceramics.

Announcements

THE first award of the Herbert E. Gregory Medal for distinguished service to science in the Pacific, established by the Trustees of the Bernice P. Bishop Museum, Honolulu, Hawaii, in memory of Prof. Herbert E. Gregory, director of Bishop Museum during 1919–36 and Silliman professor of geology in Yale University, was recently made at the tenth Pacific Science Congress to A. P. Elkin, emeritus professor of anthropology at the University of Sydney. Prof. Elkin is distinguished by his contribution to anthropological research in Australia and Melanesia and by his active role in the development of institutions supporting Pacific research.

PROF. SEYMOUR S. KETY, professor of psychiatry, Johns Hopkins University, has been awarded the Alvarenga Prize by the College of Physicians of Philadelphia for his work on cerebral blood flow and the effect of drugs on the central nervous system.

OSCAR A. QUIHILLALT, of Argentina, has been elected president of the International Atomic Energy Agency at its recent meeting in Vienna.

DR. J. W. MENTER has been appointed director of the Tube Investments Research Laboratory at Hinxton Hall, near Cambridge. The Laboratory carries out fundamental research in metal physics and physical metallurgy. During seven years at Hinxton, Dr. Menter has directed research teams studying the microstructure of metals and has promoted notable advances in the techniques of electron microscopy and X-ray microanalysis.

MR. J. C. HANBURY, chairman of Allen and Hanburys, Ltd., has been elected chairman of the British Pharmaceutical Conference for 1961–62. Mr. Hanbury took the Natural Science Tripos at Cambridge and later obtained the degree of bachelor of pharmacy of the University of London. He has spent the whole of his working life with Allen and Hanburys, Ltd.

DR. W. J. BATES, lecturer in physics at the University of Bristol, has been appointed chief engineer at R. and J. Beck, Ltd. Dr. Bates will lead Beck's design team at the Lister Works, Dickenson Street, London, N.W.5. Later he and his team will move on to the Company's new factory at Watford when it is completed.

THE Basic Science Section of the British Ceramic Society is holding a meeting on "Ceramics in Nuclear Science" at the Atomic Energy Research Establishment, Harwell, during November 28–30. Further information can be obtained from Dr. J. P. Roberts (honorary section secretary), Houldsworth School of Applied Science, University, Leeds, 2.

THE Institute of Biology is organizing a symposium on "The Better Use of the World's Fauna for Food", which will be held in the Lecture Hall, Royal Geographical Society, Kensington Gore, London, S.W.7, during October 27–28. Attendance at the symposium is open, and further information can be obtained from the Institute of Biology, 41 Queen's Gate, London, S.W.7.

A SHORT course on "Chemical Applications of Diffraction Methods" is being organized by the Department of Chemical Technology, Bradford Institute of Technology, and will be held at the Institute during November 24–25. The fee for the course is 45*s*., and further information can be obtained from the Registrar, Institute of Technology, Bradford, 7.

THE autumn meeting of the Parasitology Group of the Institute of Biology is to be held at the National Institute for Medical Research, Mill Hill, London, N.W.7, on November 10. The meeting will be devoted to a series of papers under the general title of "Parasites of World Importance". Further information can be obtained from the acting secretary of the Group, L. P. Joyner, Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, New Haw, Weybridge, Surrey.

THE South London Branch of the British Institute of Management is organizing a second conference on the preparation and use of case studies. This is to be held at the British European Airways Staff College, East Burnham, Slough, Bucks, during October 13–15. Inquiries should be addressed to Mr. B. E. Baldrey, Conference Secretary, Palmer's Travelling Cradle and Scaffold, Ltd., 3 Woodside Green, London, S.E.25.

Erratum. In the article entitled "A New Test for detecting Anaphylactic Sensitivity: the Basophil Reaction", appearing on p. 1056 of the September 9 issue of *Nature*, the fixative solution consisted of ethyl alcohol 6 ml., chloroform 2 ml., and acetic acid 2 ml. The solution did not contain acetone as stated.

UNIVERSAL LANGUAGE FOR COMPUTING MACHINES

THE traditional pattern of Section A* (Mathematics) meetings at the British Association, consisting of a division into pure and applied mathematical sessions, was this year preserved with a difference.

On September 1 at Norwich the 'pure' session opened with a paper by Prof. R. L. Goodstein having the title "Truth Tables". Starting with a simple account of formal mathematical logic Prof. Goodstein showed how the apparently 'basic' operations *and*, *or*, *not*, etc., could successively be reduced to a single pair and, eventually, to the single 'Sheffer stroke' function. The talk was a model of systematic presentation and took the audience from the elements to up-to-date research in the field of multi-valued logics.

In the discussion which followed it was made clear that this very 'pure' subject had considerable practical application, particularly as the Sheffer function gives a means for constructing the most complex digital computer from a single type of building block.

The second paper, by Prof. P. J. Hilton, was concerned with group theory and showed how the basic ideas of elementary arithmetic might better be taught via this fundamental discipline than by the present rote-learning methods.

To conclude this session, a paper by Dr. P. F. Scott described the characteristics of the various kinds of radio telescope. After showing that any large increase in size over the instrument at Jodrell Bank was impossible, he went on to show that, by using relatively small movable aerials of simple geometry, and by making the rotation of the Earth act as a scanning mechanism, the effect of receiver areas of several acres could be simulated. The catch was, that to obtain a picture of the heavens of the type used by astronomers, it was necessary to use a digital computer to process the 250,000 or so observations. Thus the first session ended with a distinct flavour of computers.

On September 5, there was a symposium on artificial computer languages. Mr. P. V. Ellis, of International Computers and Tabulators, Ltd., opened the meeting by explaining how, in the early computers, a programme was set into the machine almost by the individual operation of switches which controlled the electronic elements. He pointed out that an operation which took only a few human words to mention might take as many thousands of the code symbols understandable by a computer. From this basis he explained how the notion of a human/machine language for communication arose and described the early attempts at formulating such systems for both mathematical and business use. These were, he said, now tending to merge, but difficulties existed because, while the language of mathematics was relatively precise, that of commerce was not, so that the same words might mean quite different things to different people.

To show the three stages of interpretation which are involved, the example of developing a simple statement like : "We wish to take Smith's pay and compute the income tax", into a detailed procedure expressed in words and appropriate mathematical symbols and thence into a detailed machine coding, was analysed. After rejecting the simple solution that accountants and business men should learn mathematics—"because they will not"—current progress in the development of a commercial language 'Cobol' was described. The value of such a language, if accepted, was shown by the fact that, over the life of a machine, the cost of programming was about 1½ times that of the machine itself.

The theme of commercial computer languages was then taken up by Mr. R. M. Paine, of the Corporation for Economic and Industrial Research. After explaining that about 6,000 electronic computers of various sizes were now in operation, he went on to remark that a computer code such as :

```
        60    6    31
25      72    5    34
       400    4    17
            etc.
```

would be difficult to explain to people unfamiliar with the particular machine concerned—and possibly even to those who were. On the other hand, a statement in 'Cobol' :

```
Overtime
    If month-number is not equal to 3,
    go to print.
    If overtime rate is equal to 0,
    multiply salary by 0·015 giving overtime,
    otherwise multiply salary by overtime
    rate giving overtime,
    go to print.
```

could be understood by all.

'Cobol' was a beginning of an agreed language and was accepted by the producers of some 90 per cent of the world's output of computers. Some other attempts at artificial languages, such as the Ferranti 'Nebula', were mentioned and the paper ended with an account of a modern development whereby computers could directly understand selected words of ordinary language presented to them.

The scientific side of computer language was then taken up by Mr. C. Strachey, who suggested that programming was now emerging from its adolescent phase in which an intimate knowledge of the machine, *ad hoc* tricks, and infinite patience on the part of the programmer were of supreme importance. He suggested that the two 'new' techniques of greatest future promise were : a function language, and the replacement of the idea of linear sequences of instructions by branched chains in which no attempt was made to maintain natural order, but in which each constituent gave explicit reference to its successors. Having dismissed these topics Mr. Strachey then developed an attack on British computer designers and research workers in the universities and elsewhere. The burden of his discontent was that, whereas the American approach was to use a machine of any cost, however high, to save human effort, the British machines required the use of human genius to help the machine. He ended by claiming that this trouble was caused by the absence of very large random access stores on the British machines.

The last of the formal papers was read by Mr. R. G. Goodman, of the Brighton College of Technology.

The basic structure of the mathematical 'language' 'Algol' was explained and developed to the state in which a complete problem was coded for illustration.

The discussion was opened by Dr. A. D. Booth, of Birkbeck College, London, who, after commenting on the implication of Mr. Ellis's figure of 1½ for programming/machine cost, pointed out that Mr. Strachey's strictures were, to a large extent, unjust. The ideas of function languages were well known in Great Britain and much work has been done in them. The branched chain technique had been in use at Birkbeck College for at least three years and was described in several papers so that a claim that "no one in Britain had experience of it" was patently false. So far as lack of large, random-access, stores on British machines was concerned, Dr. Booth maintained that far from being due to lack of imagination, the situation was actually caused by lack of Government support for British machines.

A vigorous discussion, in which the topics ranged from qualifications and training of programmers to the various kinds of 'meta-language' in machine translation, ended the session. A. D. Booth

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY

By Dr. W. P. D. WIGHTMAN

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THE spate of publications relating to the Royal Society of London during the past two years seems to have increased rather than diminished the already existing misunderstanding of the relation between the Society and the famous periodical which it encouraged, but for which on several occasions the Society published or caused to be published disclaimers for any responsibility. Recently, references have been made to volumes of the *Philosophical Transactions of the Royal Society* (sometimes with '*of London*' added) in the seventeenth century, when no periodical with this title existed; statements have been made (or implied) that there was only one (or no) interruption in its publication; and, worst of all, that it was the 'official' journal of the Royal Society. The precise title would perhaps not be of any serious import but for the fact that the inclusion of the words 'of the Royal Society' suggests that it was a record of the 'Transactions' of that body and hence gives plausibility to the term 'official'. Since statements about the *Philosophical Transactions* (a few incorrect, and others by condensation definitely misleading) are scattered about over a large number of recent articles, it seemed desirable to assemble a sufficient number of 'advertisements' by the Society or its officers to establish the facts relating to the *Philosophical Transactions*. The purpose of this article is thus primarily bibliographical in the broad sense: it is not concerned with the purpose or quality of the *Transactions*. It is based on an examination (though, of course, far from exhaustive) of every volume of the *Transactions* (and *Philosophical Collections* to be referred to below) in the first two centuries of its existence.

The title on the first number issued and on the title page of every subsequent volume until Vol. 66 of 1776 was: "Philosophical Transactions: giving some accompt of the present undertakings, studies, and labours of the Ingenious in many considerable parts of the world".

There is no mention of the Royal Society other than in the imprint of its printers, John Martyn and James Allestry, and in Oldenburg's dedicatory epistle. Moreover, there is a clear indication that the editor and publisher had cast his net widely to get a representative review: the result was in fact a great deal more like *Nature* than a mere organ of publication of individual researches. But, despite this clear indication, confusion must have arisen at so early a date as to require the following disclaimer to be published in No. 12 of May 1666: "Whereas 'tis taken notice of that several persons perswade themselves that these Philosophical Transactions are publish't by the Royal Society, notwithstanding many circumstances to be met with in the already publish't ones that import the contrary: the Writer thereof hath thought fit, expressly here to declare that that perswasion, if there be any such indeed, is a mere mistake. . . . Though he denies not, but that having the honour and advantage of being a Fellow of the said Society, he inserts at times some of the Particulars that are presented to them".

The last number of the *Transactions* in the first run was dated February 1679. From some time in the same year (colophon of first number) to April 1682 their place was taken by Robert Hooke's *Philosophical Collections* "containing an account of such physical, anatomical, . . . or other mathematical and philosophical experiments and observations as have lately come to the publisher's hands. As also an account of some books of this kind lately published". A similar 'advertisement' was printed in the number of December 1681. The *Philosophical Transactions* recommenced in January 1683 with the preface: "Although the writing of these *Transactions* is not to be looked upon as the Business of the Royal Society, yet in regard they are a Specimen of many things which lie before them. . . . They have thought fit to take care for the revival thereof. . . ." Vol. 16 contained the numbers issued during 1686 and 1687 and bore the imprint "London 1688" (some previous volumes were printed at Oxford "at the [Sheldonian] Theater"). Thereafter, publication was suspended until early in 1691 "chiefly by reason that the unsettled posture of publick affairs did divert the thoughts of the curious towards matters of more immediate concern than are physical and mathematical enquiries. . . ." These were, of course, the years of the 'Glorious Revolution', and this gap in the *Transactions* has been overlooked in some accounts.

It is not possible, nor indeed necessary, to cite every declaration of editorial policy: all call the same tune with variations—a review of what is going on in the world of 'philosophy', including "an exact account of such experiments as having been made before the

illustrious Royal Society . . . they shall be pleased to order or permit the publication of" (Halley, 1714).

Then in the Volume 47 (London 1753, but as usual containing the numbers issued during the years 1751 and 1752) came the announcement of a fundamental change: "The Committee appointed by the Royal Society to direct the publication of the *Philosophical Transactions*, take this opportunity to acquaint the public, that it fully appears, as well from the Council-books and journals of the Society as from the repeated declarations which have been made in several former *Transactions*, that the printing of them was always, from time to time, the single act of the respective Secretaries, till this present XLVII volume. And this information was thought the more necessary, not only as it has been the common opinion, that they were published by the authority, and under the direction, of the Society itself; but also, because several authors, both at home and abroad, have in their writings called them the *Transactions of the Royal Society* [italic in original]. . . .

"But the Society being of late years greatly inlarged . . . it was thought adviseable that a Committee of their Members should be appointed to reconsider the papers read before them, and select out of them such, as they should judge most proper for publication in the future *Transactions*, which was accordingly done upon the 26 of March 1752."

In 1752, then, for the first time the *Philosophical Transactions* were in fact, but not yet in name, "of the Royal Society": the old, and by then quite unsuitable, title was retained until Vol. 66, dated 1776.

That the announcement of this change of policy was not a mere formality is shown by reference to the Council minutes for January and February 1752, when a number of 'questions' put by Lord Macclesfield (not yet president) were considered. The only one relevant to our purpose was whether there was any evidence of "intermeddling" by the Society with the *Transactions*, to decide which Thomas Birch, secretary, was requested to take the relevant literature home and examine it. Within a week he had made a sufficient investigation to satisfy the Council, and the changes were made as already described.

It remains only to add that the announcement made in 1752 appeared regularly for the next 123 years: it appeared for the last time in 1875, by which time it was no longer true that "in late years" the Society had been "greatly inlarged"; it had, on the contrary, in pursuance of the 'reforms' of 1847, been greatly reduced.

DEOXYRIBONUCLEIC ACID: STRUCTURE, SYNTHESIS AND FUNCTION

THE eleventh Annual Reunion of the Société de Chimie Physique was held at the Col de Voza during June 26–30 and was devoted to papers and discussions on the structure, synthesis and function of deoxyribonucleic acid (DNA). There were some thirty contributors to the symposium and the total number of participants was about 100. The full texts of the contributions, including the discussions, are to be published in the October and November issues of *Journal de Chimie Physique* and will be republished afterwards in the form of a book.

The papers were divided into groups according to their subject-matter and the meeting commenced with a session on "Macromolecular Structure of DNA". Ch. Sadron (Strasbourg) presented results pointing to the existence of sub-units in DNA particles in solution and this was followed by a paper from C. Levinthal (Cambridge, Mass.) on the hydrodynamic shear degradation of *T2* bacteriophage DNA in solution. This degradation of DNA can be brought about by normal laboratory procedures, and this had led to the conclusion that the molecular weight of DNA in cells is probably very much greater than the 6–10 million usually quoted and might be as much as 100 million. M. H. F. Wilkins (London) presented new data on the *A*, *B* and *C* configurations of DNA and evidence against the existence of base pairing other than that of Watson and Crick. In addition, the structure of denatured DNA and the relation of such structures to RNA was discussed. X-ray diffraction studies on DNA in solution were reported by V. Luzzati (Strasbourg), who concluded that the form and molecular weight of DNA were the same in dilute solution and in gels.

The second session dealt with "Electronic Aspects of Nucleic Acid Structure" and started with a paper by B. Pullman (Paris), who dealt with the electronic structure of nucleic acid constituents and the influence of this on the chemical and physico-chemical properties of DNA. This was followed by a paper from M. Kasha (Tallahassee), who was concerned with $n \rightarrow \pi^*$ optical transitions in polynucleotides, the exciton splitting phenomenon in hydrogen-bonded *N*-heterocyclic base pairs, and energy transfer and hypochromism in polynucleotides. L. A. Blumenfeld (Moscow), who unfortunately was not present, submitted an abstract on the magnetic and dielectric properties of DNA, and P. Douzou (Paris) presented results of experiments on the excitation and emission spectra of purine and pyrimidine bases, the corresponding mononucleotides, polyadenylic acid, DNA and nucleoproteins.

The third session was entitled "Chemical Sequence and Heterogeneity", and commenced with a presentation by B. Horecker (New York) of some recent results of Hurwitz and his collaborators on the DNA-requiring system for polyribonucleotide biosynthesis. In this system, the base composition of the newly formed polyribonucleotide appears to be determined by that of the DNA primer. J. Marmur (Brandeis University) described in some detail work on strand separation in DNA, on the recombination of strands and on the formation of hybrid chains. A system capable of the non-enzymic synthesis of polydeoxyribonucleotides from mono- and oligo-nucleotides present in deoxyribonuclease digests of DNA was described by A. Bendich (New York).

"Enzymic Synthesis of DNA" was the title of the fourth session, which was opened by a paper from R. M. S. Smellie (Glasgow) on the properties and intracellular location of the DNA polymerase in certain mammalian systems and on some possible mechan-

isms of control of the biosynthesis of DNA. This was followed by a presentation by J. A. V. Butler (London), who described some experiments on the phosphorylation of thymidine by tissue extracts and on the incorporation of labelled thymidine into DNA. S. S. Cohen (University of Pennsylvania) discussed the changes in enzyme complement of bacteria infected with *T*-even bacteriophage, and referred in particular to the appearance of an active system which converts ribonucleotides to deoxyribonucleotides. Some recent work on the mechanism of replication of bacteriophage φ*X*174 were described by R. L. Sinsheimer (Pasadena), from which it seems that on infection a replicative form of DNA is produced which has characteristics of a double-stranded DNA.

The fifth and sixth sessions dealt with "Induced Changes in DNA". R. Litman (University of Colorado) discussed the effects of ultra-violet irradiation and nitrous acid on pneumococcal-transforming DNA, and the chemical and biological effects of nitrous acid and hydroxylamine on DNA from tobacco mosaic virus and *T*-even bacteriophages were described by H. Schuster and W. Vielmetter (Tubingen). P. D. Lawley (London) reported on the effects of alkylating agents on DNA. The relative reactivities of the different bases in DNA were described and the possibilities of cross-linking in the DNA molecule were discussed. Experiments directed towards elucidating the mechanism of action of succinic peroxide on DNA by chemical, physico-chemical and biological procedures were discussed by D. Luzzati (Strasbourg). G. Stent (Berkeley) discussed the susceptibility to mutagenesis by 5-bromodeoxyuridine and the stabilization to phosphorus-32 decay-inactivation of *T*4 bacteriophage-infected bacteria. D. Shugar (Warsaw) was unable to attend the meeting, but submitted an abstract dealing with the primary effects of ultra-violet radiation on nucleic acids; these include hydration and dimerization of pyrimidine residues. An additional paper on the dimerization of thymine by ultra-violet radiations was presented by W. Berends (Pays Bas). R. Latarjet (Paris) described recent investigations on the action of X-rays on pyrimidine bases in aqueous solution with the formation of hydroperoxides, and some work on the lethal effects of radioactive decay in bacteria after labelling of the DNA in different sites with phosphorus-32, carbon-14 and tritium. W. Harm (Cologne) discussed the reactivation of bacteriophage following inactivation by exposure to ultra-violet light and divided the reactions into two principal classes, namely, 'recombinative reactivation' and 'true repair mechanisms'.

The seventh session was concerned with "Code and Replication". A. Garen (Philadelphia) described alterations in alkaline phosphatase resulting from mutations, and J. Weigle and F. Stahl (Pasadena) discussed chromosome breakage and genetic recombination in bacteriophage λ and the role of duplication in recombination.

"Genetic Aspects" provided the subject of the final session of the symposium. L. Grossman (Brandeis University) presented some work on the chemistry and antigenecity of DNA from *T*2, *T*4 and *T*6 bacteriophage. Thermally denatured DNA from these bacteriophages will react with antibodies in the antisera of rabbits immunized with the respective ruptured bacteriophage, but native or renatured DNA shows little reactivity. H. Ephrussi-Taylor (Paris) described a study of recombination at the molecular level in bacterial transformations and F. Jacob and F. Gros (Paris) discussed the regulation and mode of action of genes. The role of messenger RNA in these reactions was also discussed. The final paper of the session was delivered by R. Herriott (Baltimore), who described experiments on the annealing of mixtures of transforming DNAs.

The timing of the meeting was such as to allow for plenty of discussion during each session, and advantage was taken of this by many of the participants with the result that lively and valuable discussions followed most of the presentations. In every respect this was a highly successful meeting and the organizing committee of the Société de Chimie Physique is to be congratulated on the arrangements.

R. M. S. Smellie

QUANTITATIVE BIOLOGY

An informal symposium on "Quantitative Biology", at which sixty biochemists, biophysicists and biologists met to discuss their common problems, was held at the Dominion Physical Laboratory, Lower Hutt, New Zealand, on May 12. The meeting was arranged by the biophysicists, who felt that New Zealand workers in these fields had insufficient opportunity to get together. The enthusiastic response and the success of the meeting showed how general this feeling must have been.

In the morning session of the symposium the hosts introduced their own work. Dr. M. C. Probine, who is measuring the physical properties of the primary wall of plant cells, showed how changes in the shape of growing *Nitella* cells could be predicted from the initial orientation of the reinforcing microfibrils and the elastic and plastic properties of the wall. Dr. K. J. McCree discussed his programme for measuring the photoconductivity of oriented monolayers of organic materials, produced by the Langmuir technique. Mr. M. A. Collins described his electron-spin-resonance spectrometer, and demonstrated the signals of free radicals occurring naturally in coal and those produced by the γ-irradiation of cellulose. The spectrometer will also be used to study the physics of photosynthesis, in conjunction with the photoconductivity experiments.

Some striking electron micrographs of the wax on the surfaces of clover leaves were exhibited by Mr. D. M. Hall. They showed how the pattern varied with the species of clover, and how the wax could be removed by the rubbing together of leaves in a high wind. Mr. W. S. Bertraud described the changes in the components of the spermatid cells of the cave weta (*Pachyrhamma fascifer*) which took place during spermiogenesis.

In the afternoon, Dr. E. G. Bollard, of the Fruit Research Division, Department of Scientific and Industrial Research, described the utilization of various organic nitrogen compounds as sole sources of nitrogen, in the sterile culture of *Neurospora*, *Chlorella*, *Spirodela* and carrot root tissue. He was followed by Dr. R. E. F. Matthews, of the Plant Diseases Division, who had found (working with

Dr. R. I. B. Francki) that the uracil analogue 2-thiouracil had very different effects on the rod-shaped tobacco mosaic virus and on the spherical turnip yellow mosaic virus. After tea, Dr. G. A. Nicholls, of New Zealand Forest Products, Ltd., took his audience on an illustrated tour of his firm's plant, and discussed the work of his laboratory in the fields of bark utilization, pulping, bleaching and turpentine refining. Dr. A. T. Wilson, of Victoria University of Wellington, then wound up the proceedings with a highly entertaining exposition of his theories on the physical system in which life may have originated.

In the discussion of the way in which future meetings should be organized, there was general agreement that they should be kept as informal as possible, so that ideas could be aired and discussed before they had reached the stage of publication. It was also agreed that the meetings should be held at various laboratories in turn, so that in time everyone would have a first-hand knowledge of what everyone else was doing. Of the various time-intervals which were suggested, a year was most favoured, and Drs. McCree and Probine were asked to handle the arrangements for the next meeting. K. J. McCree

MEDICAL RESEARCH IN THE BRITISH CARIBBEAN

THE sixth scientific meeting of the Standing Advisory Committee for Medical Research in the British Caribbean was held at the Marine Hotel, Bridgetown, Barbados, during April 10–13. Thirty-eight communications were delivered, and six were read by title only. Chairmen for the five sessions were Sir Harold Himsworth, Prof. T. H. Weller, Prof. A. C. Frazer, Dr. E. T. C. Spooner and Prof. E. K. Cruickshank.

The meeting was remarkable for the high standard of presentation, the diversity of subject-matter, and for the evidence which it offered of the stimulating effect of a research interest on all fields of medicine and its ancillary professions. The theme of the meeting—the impact of research on public health—was chosen to be as nearly all-embracing as possible.

R. A. Irvine, C. Miller and L. S. Grant (Univ. Coll., West Indies) reviewed typhoid infection in Jamaica. During the period 1952–59, typhoid fever had affected chiefly the younger age groups, with a relapse-rate of 8 per cent and a case fatality of 5·8 per cent. M. T. Ashcroft (British Guiana) reported a steady decline in fatality of typhoid fever in British Guiana, partly due to improved water supply and partly to the introduction of chloramphenicol treatment. C. C. Nicholson and J. M. Ritchie (British Guiana) directed attention to imperfections still remaining in British Guiana water supplies. E. H. Back and S. E. Brooks (Univ. Coll., West Indies) analysed 134 cases of gastroenteritis in infants admitted to University College Hospital, Jamaica, and discussed prognosis. F. C. Ramsey (Barbados) described scarlet fever in Barbados, suggesting that the disease is less rare than it is said to have been. G. Bras, D. Watler and S. E. Brooks (Univ. Coll., West Indies) quoted figures from the Cancer Registry at University College Hospital to show that carcinoma of the cervix in Jamaica is responsible for more than 30 per cent of all malignancy in females. J. D. Arneaud (Trinidad) discussed drug resistance of tubercle bacilli isolated in Trinidad. K. D. B. Charles (Jamaica) and L. S. Grant (Univ. Coll., West Indies) reviewed poliomyelitis in Jamaica with special reference to the epidemics of 1954, 1957 and 1960. J. S. R. Golding (Univ. Coll., West Indies) described the management of those disabled in a moderately severe epidemic of poliomyelitis, and demonstrated the necessity for a planned programme. L. Spence (Trinidad) gave an account of the distribution of Eastern equine encephalitis virus in Trinidad and British Guiana. In Trinidad, the virus has been isolated on six occasions from culicine mosquitoes; and in British Guiana from horses during an outbreak. In Trinidad, immunity has been demonstrated in humans, birds and poultry. L. S. Grant (Univ. Coll., West Indies) reported a survey of *Q* fever antibodies in Jamaica, showing that this infection is not at present prevalent. W. G. Downs, L. H. Turner and A. E. Green (Trinidad) found 10 per cent of human sera from cases of undiagnosed fever in Trinidad agglutinated leptospiræ, and reported 17 isolations of leptospiræ in culture. They directed attention to the lack of knowledge of the local ecology of leptospirosis. A. V. Wells (St. Lucia) described the successful eradication of malaria from St. Lucia. G. Giglioli (British Guiana) discussed the effect of malaria eradication in British Guiana on mortality from chronic nephritis. F. R. S. Kellett (Trinidad), as part of the malaria eradication plan in Trinidad, had used 'Baytex' (*S* 29493) as a spray, and showed that this insecticide is effective on various household pests including culicine mosquitoes. T. H. G. Aitken (Trinidad) discussed the ecology of *Culex quinquefasciatus* and its importance for public health in the Caribbean area as a vector of filaria, St. Louis encephalitis and Cache Valley fever. L. M. Burke (Jamaica) gave an account of the clinical features of poliomyelitis in Jamaica, as seen in a study of 309 cases admitted to Kingston Public Hospital during 1957–60. J. A. S. Hall (Jamaica) discussed the role of infection in a series of twenty cases of myelomatosis. E. H. Back and E. E. DePass (Univ. Coll., West Indies) considered rheumatic fever as seen in University College Hospital, Jamaica, during 1952–60, and described the beneficial effect of maintenance benzathine penicillin. F. M. McWatt (British Guiana) read an interesting paper on liver cirrhosis in cattle on the savannahs of British Guiana, where three species of *Crotalaria* have been identified. The cause of the disease has not been ascertained, but liver biopsies have shown that the fibrosis regresses at certain times of year or when the animals are moved to improved pastures. E. E. Ward and E. H. Back (Univ. Coll., West Indies) reported work on electrolyte disturbance in infantile gastroenteritis. T. Poon-King (Trinidad) gave an important account of electrocardiographic changes observed in patients stung by the Trinidad scorpion, *Tityus trinitatis*. The sting of this scorpion produces severe physiological disturbance and sometimes kills. Electrocardiographic abnormalities were found in 39 of 45 patients studied. E. E. Ward (Univ. Coll., West Indies)

discussed γ-globulin-levels in Jamaicans; he found no racial differences, but levels were higher in those with chronic infections than in the healthy. R. A. Webb (Barbados) analysed 330 first admissions to the Barbados Mental Hospital, in which records of mental ill-health for the whole island have been kept for many years. Heredity was revealed as a more important etiological factor than environment. K. L. Standard (Barbados) presented an analysis of child mortality in Barbados, reporting a marked reduction in infant mortality over the past six years. Miss G. Shaw, L. S. Grant and A. A. Peat (Jamaica) gave an account of the statistics of communicable disease in Jamaica up to 1956. K. H. Uttley (Antigua) discussed mortality from tuberculosis in the negro population of Antigua over the past 100 years. B. Weinstein (British Guiana) reported a survey of diabetes among residents on the sugar estates of British Guiana. He found an overall incidence of 2·04 per cent and discussed etiological factors. J. Parker-Williams (Univ. Coll., West Indies) described a survey of abnormal hæmoglobin in Barbados, with special reference to the sickling gene. He compared Barbados with Jamaica in this respect, and discussed genetical implications. R. J. Gourlay (Univ. Coll., West Indies) described the incidence of certain worm infestations in Jamaica. Miss A. Walters and Mrs. G. Jordan (Barbados), two public health nurses in Barbados, presented a most interesting time-study of the work of public health nurses at three health centres in Barbados, showing that more time was spent on maintenance and records than on the treatment and care of patients. H. M. Beadnell (British Guiana) recounted eight years experience with injuries and workmen's compensation in the sugar estates of British Guiana. D. O. Gore (Univ. Coll., West Indies) gave a clinical and pathological account of the portal hypertension produced by 'veno-occlusive disease', and reported on six patients treated by establishing porto-caval shunts. F. F. Laband (Trinidad) gave an account, illustrated with colour slides, of four years experience of oral surgery in the San Fernando General Hospital, Trinidad. A. G. Leacock and R. K. Rowley (Barbados) reported the results of repairing inguinal hernias by the 'nylon darn' technique. J. A. B. Jones (Univ. Coll., West Indies) discussed some anatomical and pathological abnormalities of the alimentary tract as seen in Jamaica. Winston Adams (British Guiana) gave an account of the control of two malaria outbreaks in the interior of British Guiana, discussed the difficulties of control of *Anopheles darlingi*, the local malaria vector, and mentioned the programme of chloroquinized salt prophylaxis now in operation in this area. E. L. S. Robertson (Trinidad) showed a film demonstrating the surgical treatment of filaria lymphœdema.

Excellent arrangements were made for the meeting by Dr. Maurice Byer, director of Medical Services, Barbados, and his staff.

HISTORY OF SCIENCES IN ANCIENT AND MEDIEVAL INDIA

A SYMPOSIUM on the "History of Sciences in Ancient and Medieval India" was held during August 4–5 at the Bose Institute, Calcutta, under the auspices of the National Institute of Sciences of India. The symposium was inaugurated by Dr. D. M. Bose, director of the Bose Institute. In his opening remarks, Dr. A. C. Ukil, convener of the symposium, referred to the importance the study of the history of sciences in India had assumed during recent years and the problems which the workers in the field have to face, especially in connexion with the source materials. The discrepancy of knowledge regarding chronology, he observed, constituted a serious difficulty which should engage the attention of the research workers in the subject. Regarding the investigations already made, which he termed secondary sources, the attitude taken in the past and points of view expressed required in many instances to be re-examined and re-evaluated.

Thirty-five papers were received for the symposium of which twenty-three were read and discussed. In his paper on the social and international relations in the development of science, Dr. R. C. Majumdar pointed out that there is direct evidence to show that scholars from India visited Western countries and taught the people there medical science, astrology, astronomy, algebra and arithmetic as known to India and also indirect evidence of international exchange of scientific knowledge between India and foreign countries. European scholars have generally held that in scientific matters it was India that must have borrowed from Western countries. But with the advance in knowledge of history, he observed, it was difficult to subscribe to this view of one-way traffic. This observation was further substantiated in the paper by Sri S. N. Sen, who furnished archæological and epigraphic examples and also case-histories such as the transmission of the knowledge of the decimal place-value notation, and some medical and astronomical ideas.

In biology, health and medicine, Dr. N. H. Keswani discussed the concepts of generation, reproduction, evolution and human development as found in the writings of Indian scholars of the ancient and medieval period. He noted that the results of their observations, unaided by microscopes and modern appliances, approached in accuracy those of modern work, especially in the field of embryology. Zoological knowledge in the works of Kâlidâsa formed the subject of two papers by Sri C. S. Gupta and Sri B. Rama Rao, while Dr. S. S. Misra gave a review of the development of medical sciences in India.

On Indian chemistry, Dr. B. V. Subbarayappa's paper dealing with Indian atomism showed independent development of the atomic concept of matter in the Hindu, Buddhist and Jaina literature and the difference between the Indian ideas and those propounded by the Greek scholars headed by Democritus and Leucippus. Dr. S. P. Raychaudhuri and Dr. A. K. Bhattacharyya dealt with the agricultural practices and soil science in ancient India.

The geographical ideas of the Hindus were presented by Dr. S. M. Ali. Besides these were the discussions on the development of mathematical series in India after Bhâskara II, Bhâskara's works on differential and integral calculus, science and society in the Arthaśâstra and the Râmâyaṇa, and the general scientific contents in the Vedic literature. S. N. S.

TELEVISION FOR SCHOOL SCIENCE

THE economic strength of Western Europe depends increasingly on the application of science and technology and, consequently, on a growing supply of scientific and technical manpower. Unfortunately, there is a growing shortage of teachers and schools are already overburdened. School television could perhaps be of considerable assistance in overcoming such deficiencies in educational facilities. The medium has proved effective in various countries, including France, Italy, the United Kingdom and the United States of America, while its scope has been considerably extended by recent technical developments such as the use of closed-circuit systems. A year ago, the Organization for European Economic Co-operation, believing that much could be gained from the experience of countries which had been exploiting the new medium for teaching science subjects, conducted a survey on the present status of school television in Western Europe, and convened a forum of international experts in this field, "Teaching through Television", during June 1960. The report of this meeting is now available*.

The seminar was held at Ashridge (Nr. Berkhamsted), with three main objectives: to give countries beginning educational television an opportunity to learn from the experience of those where educational television has been in operation for some years; to encourage the development of international contacts, on a personal basis, between programme producers and to seek ways and means to secure improved co-ordination in securing sources of information on programme material. Two different tendencies emerged during the conference: the use of school television as a complete substitute for traditional teaching (this is the case with the 'Telescuola' in Italy, where there is a severe shortage of teachers and schools) or as a complementary broadcast for use in the framework of normal school activities. School television is also used for teachers' training, as, for example, in the 'continental classroom' of the United States.

Other topics included technical aspects of programme-production problems, the use of films, of consultants, advisers and specialists, of economical visual techniques and devices. Future developments were concerned chiefly with the use of closed-circuit television. Great progress has been made in the development of inexpensive systems which can be used to link any number of classrooms (or even schools) to a central school studio from which live or recorded lessons are 'piped' to pupil audiences. During the seminar it became clear that great possibilities exist for the exchange of information on films and other material and the international exchange of production facilities and personnel.

The report also provides information about the Tele-Lille mathematics experimental project, lists of films available for educational television, examples of science programmes, and a list of scientific film associations and their addresses. A small group has been set up to carry out the recommendations (improved information, training school science producers, research, experimental projects, co-operation with industries) of the seminar. It will report progress to the Organization for European Economic Co-operation and ensure liaison with organizations concerned with science teaching, films and television.

* Organization for European Economic Co-operation: Office for Scientific and Technical Personnel. Television for School Science: Report on an O.E.E.C. Seminar, Ashridge (England), July 1960. Pp. v + 181. (Paris: Organization for European Economic Co-operation, 1961.)

THE INSTITUTE FOR THE ENCOURAGEMENT OF SCIENTIFIC RESEARCH IN INDUSTRY AND AGRICULTURE, BRUSSELS

THE annual report of the Institute for the Encouragement of Scientific Research in Industry and Agriculture, Brussels, for 1960* records sixty subsidies, totalling 264,772,837 francs, during the year, including 3 million francs to the Committee for the Study and Exploitation of Electronic Computers, and estimates that 267 research workers and 529 technicians are thereby employed. The National Centre for Metallurgical Research received 29,275,000 francs for analytical research, investigations on the physical metallurgy of steel, analytical research on the physical metallurgy of zinc, research on the agglomeration of minerals, etc., while the Belgian Society for Nitrogen and Chemical Products, of Marly, received 25,898,000 for research on the pyrolysis of hydrocarbons, on the synthesis of vinyl chloride and other investigations in the field of heterogeneous catalysis.

The Committee for Mapping the Soils and Vegetation of Belgium received a grant of 20,155,000 francs for its systematic study of the pedological character of the soils and towards the pedological map of Belgium, while grants of 10,000,000 francs and 8,560,000 francs went to the Centre for the Study of High Polymers for its work on high polymers and their degradation, on photopolymerization initiated by silver salts and the stability of the polymers and for some industrial work on the extrusion of crystallizable polymers. The Centre of Applied Electronics received 12,450,000 francs for work on passive circuits, on amplifiers, modulators and oscillators of transistors and on industrial circuits; 11,505,000 francs went to the Committee for the Study of Diseases and Feeding of Farm Animals; 9,950,000 francs to the Centre for Scientific and Technical Research of the Metal Manufacturing Industry for investigations on the adaptation of numerical techniques to other machine tools and in automation, as well as 2,980,000 francs for research on resistance to fatigue of cooled alloys, etc.; 8,317,590 francs to the National Centre of Metallurgical Research for low-temperature furnace research, and 7,474,273 francs to the Scientific and Technical Centre of the Belgian Textile Industry for research on wool, cotton,

* Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel Exercice, 1960. Pp. 255. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1961.)

jute, linen and rayon, as well as 1,534,000 francs for investigations of the utilization of acrylic fibres.

A grant of 7,050,000 francs went to the Committee for the Study of the Solid State for continuation of work on the mechanism of photographic processes and of sensitization; 6,520,000 francs to the National Committee for the Study of Fruit Cultivation; 5,360,000 francs to the Committee for the Scientific and Technical Study of Milk and its Derivatives; 4,300,000 francs to the National Centre for Research on Herbage and Forage for botanical, phytosociological and ecological studies on herbage and on the control of adventitious plants of the prairies; 4,050,000 francs to the Centre for Scientific and Technical Research on the Conservation of Vegetables; and 3,817,000 francs to the Belgian Centre for the Study and Documentation of Water, for studies on water supply, corrosion, residual water, and on atmospheric corrosion.

The Belgian Institute of High Polymers received a grant of 3,710,000 francs towards its experimental thermodynamic work at high pressures on the inhibition of the explosiveness of acetylene; the Centre for Studies and Research in Agriculture, 3,590,000 francs for research on cultivation in mineral substrates and on the mineral requirements of vegetables; the Scientific and Technical Centre of the Brewing, Malt and Related Industries, 3,316,000 francs; and the Committee for the Study of the Structure of the Soil, 3,000,000 francs. Among other grants may be mentioned 2,770,000 francs for studies on diseases of cereals; 2,280,000 francs for ceramic research; 2,125,000 francs for research on the synthesis of saturated heterocyclic amines substituted in position 3 or 4; 2,065,000 francs for phytovirological research; 2,049,500 francs for research on paints, varnishes and printing inks; 2,040,000 francs for studies on the creep of metals at high temperatures; 2,000,000 francs for work on new penicillins and the new antibiotic, staphylomycin; and 1,840,000 francs for research on the culture of medicinal plants.

HELICOPTER STATIONS IN LONDON

IN July 1959, the Minister of Transport and Civil Aviation formed a committee to consider the setting up of helicopter stations in the London area, assuming: (1) that the helicopter is developed to the point of offering sufficiently low fares on regular services to attract large numbers of passengers for inter-city flights on international routes and possibly internal ones as well; (2) that the helicopter will not offer fares sufficiently low for mass travel, but will meet the needs of small numbers of travellers for whom the extra time saved is more important than the money spent.

In its recommendations*, the Committee assumes that regular services between London and the near Continental cities with helicopters carrying twenty-five passengers or more will begin in 1965, or soon after, that fares will be offered approximating those of first-class 'fixed-wing' services, and that services to provincial cities in the United Kingdom will follow if fares can be reduced to compete with those of the other forms of transport.

Allowing for executive and charter flights with passengers, freight and mail as well as regular services, the total traffic could amount to 20–25 helicopter movements in a peak hour, with a mixture of single- and multi-engined types by 1975, when the heliport would have to handle 1½ million passengers a year. These estimates, the committee suggests, represent a reasonable basis for planning the area required for the first permanent heliport.

In order to provide enough space for helicopters to land and take-off and for working stands (for loading, unloading, minor maintenance, etc.) at the heliport, a level area of about 8 acres would be required to handle 25 movements an hour. In addition, an area of about 4 acres would be needed for terminal buildings and car parks, which, if the landing area were on a raised platform, could be provided on a lower deck or at ground-level.

For reasons of air traffic control, the capacity of a heliport could not be increased much beyond 25 movements an hour even by providing an additional landing area, which would involve a disproportionate amount of extra space.

The heliport should satisfy the following requirements:

(1) Since the appeal of the helicopter depends on saving time, the heliport should be brought as close as possible to the main source and destination of a substantial proportion of the traffic. More specifically, it should not be much more than a quarter of an hour from Grosvenor Square, and ideally, between the West End and the City. Because of the helicopter's potentialities for freight and mail, nearness to one of the sorting offices of the General Post Office would also be an advantage.

(2) It should be conveniently placed for all forms of surface transport, good road connexions and local traffic access being especially important in view of the high proportion of passengers who will travel to the heliport in private cars and taxicabs.

(3) It should have sufficient unobstructed approaches to enable operations to continue safely in all weathers, allowing for a climb and descent gradient not much steeper than 1 in 4. This may entail some restriction on building heights in the neighbourhood of the site.

(4) To be usable at all by single-engined helicopters it should be beside the River Thames.

(5) In order to avoid conflict between helicopter and fixed-wing traffic to and from London Airport, it should not be located farther west than Battersea. For effective control of air traffic, it is unlikely that two sites on the Thames within easy reach of the West End could be simultaneously used to capacity.

(6) It should be compatible with the proposals of the County of London development plan.

(7) It should be so placed as to avoid interference with the normal life and work of the neighbourhood.

Measurements of the noise of the various types of helicopters indicate that noise in level flight at 1,000 ft. would be no greater than traffic noises in a busy street, but, since it will be more widespread, routes should be chosen which, so far as possible, reduce flights over areas particularly sensitive to noise. The greatest noise will be in the immediate locality of the

*Ministry of Aviation. Report of the Committee on the Planning of Helicopter Stations in the London Area. Pp. 105 (10 plans). (London: H.M.S.O., 1961.) 7s. net.

heliport, and within a radius of half a mile of it under the take-off and landing paths.

Provided the site is carefully chosen, that is, on the Thames and in an area where the activities are mainly industrial and commercial, the quieter types of turbine-driven helicopter, as well as existing piston-engined types, should be able to use it at moderate frequencies without causing a serious problem. Whether the same would apply to high-frequency or night operations remains to be seen. Furthermore, tests carried out in open country and over London suggest that interference with television and very-high-frequency broadcasting is unlikely to be serious.

A continued effort will be needed to clarify the effects of the noise of helicopters and means of abating it. Research into noise abatement at source is being carried out by Government establishments and manufacturers. Meanwhile, proposals for heliport development should be expedited on the basis that noise can and will be brought down to reasonable levels.

After a preliminary survey, nine sites near the River within reasonable range of the West End have been examined and the following locations are considered the most promising: Nine Elms; Cannon Street Station; St. Katherine Docks.

No attempt has been made to assess precisely the cost, since this is a matter for the promoters. The capital cost is likely to run into several millions of pounds, although it may vary considerably between one site and another. A single heliport of 12 acres should be capable of handling traffic well into the 1970's, but will not be able to handle all the traffic indefinitely if the helicopter becomes accepted as a means of mass travel. The question of a second heliport must be left for a decision until nearer the time when the requirement is seen to emerge.

THE RED DEER IN SWEDEN

FROM information furnished by Å. Sjöström, in a bulletin from the Royal School of Forestry in Stockholm, it appears that the red deer in Sweden is now reduced to some 150 animals confined to three relatively isolated localities in the southern province of Skåne*. The paper gives the results of a study conducted over an area of about 740 acres within the Sövdeborg locality, which extends to some 4,300 acres and contains a herd of 30 deer. The annual hunting licence allowed the shooting of 5 animals but might be extended to equal the annual increase, which is 8. The area studied contains some 376 acres of Norway spruce plantations and 179 acres of beech forest, to which the deer do a considerable amount of damage, said to be due to a deficiency of certain nutrient elements in their diet. A careful assessment is made of the damage, which is caused mainly to trees from 15 to 45 years old, and leads to the conclusion that the cost of producing one red deer is 4,000 kr. (£280). In a Danish deer-forest of some 7,200 acres with a head of 60–100 animals, Prof. Grøn has estimated that the cost of production of one animal is about 6,700 kr. (£465). Among the suggestions for dealing with these remnants of Sweden's original fauna are fencing of plantations—regarded as too expensive; change of tree-species—thought to be a possible solution; use of chemical repellents—regarded as of doubtful and uncertain efficiency and too expensive; heavier shooting—inapplicable in view of the already small number of animals; formation of red deer reserves and, finally, transfer to more suitable localities in natural spruce forests in west and central Sweden. One such transfer has already started. The interesting proposal is made that a charge of 10 kr. (14*s*.) per head on every roe deer, for which a shooting licence is required, should be levied. This would bring in about 300,000 kr. (£20,800) a year, which would be used to purchase and maintain an area of suitable land where a new and vigorous stock of the Swedish red deer could be built up.

* Kungl. Skogshögskolans Skrifter. (Bulletin of the Royal School of Forestry, Stockholm.) Nr. 35: *Kronhjortens Skadegörelse på Granskog: Examensarbete vid Skogshögskolan i ämnet Skogszoologi med Viltvård Utfört ar* 1959. Av Åke Sjöström. Pp. 36. (Stockholm: Skogsbiblioteket, Skogshögskolan, 1961.) 5 kr.

O'NYONG-NYONG FEVER: AN EPIDEMIC OF VIRUS DISEASE IN EAST AFRICA

EARLY in 1959 an outbreak of unfamiliar disease appeared in human populations in the north-west corner of Uganda, and spread across the northern half of the country in a south-easterly direction. The disease was usually characterized by headache, fever, adenitis and crippling joint pains, frequently followed on about the fourth day by an eruption of an itching rash, mainly on the trunk and arms. These symptoms usually persisted for 5–7 days and were generally followed by quick and complete recovery.

The clinical, and some of the epidemiological, aspects of the disease were investigated by Dr. H. Shore, the acting provincial medical officer in the Northern Province of Uganda, who reported that a dengue-like disease was affecting "vast numbers of people, old, young, men, women and children", and provisionally estimated from the rate of attack that at least 25–50 per cent of the population was at risk. That all ages were affected led him to suspect a causative agent new to the region. This view is supported by the fact that the local African population have coined a new name for the disease in each successive language group in which it has appeared, one of the first of these being 'o'nyong-nyong'.

The epidemic advanced steadily on about a 100-mile front for ten months, crossing the Uganda–Kenya border some 300 miles south-east of the original site of the disease in Uganda, in November, 1959. Between December 1959 and February 1960, the epidemic fanned out in the Kano Plains near Kisumu, Kenya. It is still active at discontinuous points east, south and west of Lake Victoria. From June, the epidemic has been under detailed study by members of the East African Virus Research Institute Staff, in close collaboration with members of the Uganda, Kenya and Tanganyika Medical Departments.

In a report*, prepared by J. D. Gillot, M. C. Williams, J. P. Woodall, P. S. Corbet and J. M. Ellice, it is shown that the isolation of o'nyong-nyong

* East Africa High Commission. East African Virus Research Institute Report, July 1959–June 1960. Pp. i + 57. (Entebbe: East African Virus Research Institute, 1960.)

virus allowed field epidemic studies and attempted transmission work to be followed virologically, and opened the way for quantitative investigations. These support the general epidemiological and vector findings that *Anopheles* species and, in particular, *A. funestus* are responsible for the transmission of o'nyong-nyong fever, but they do not exclude the possibility of transmission by *A. gambiae*. It is thought that this is the first time that a patchy alopecia has been used as an indicator in mice of arthropod-borne virus infection. An attempt has been made to measure three of the variable factors involved in order to gain an understanding of the epidemic. These are the mosquito infection-rate, the inapparent human infection-rate and the period of viræmia. Considerable work will be required before the success or failure of this attempt can be assessed.

A WARNING SYSTEM FOR NUCLEAR ACCIDENTS

RADIOACTIVITY, which was once the practical interest of no more than a few scientists and medical men, is now the daily concern of a great many people in most industrialized communities. Moreover, as events at Windscale demonstrated in Great Britain, radioactivity can be released accidentally in quantities sufficient to affect the life and livelihood of considerable numbers of the general population. With the increasing use of nuclear energy for peaceful purposes, nuclear installations will become more numerous, and, in the event of a nuclear accident, radioactive material might be transmitted over wide areas and across national boundaries.

The decision of the Council of the Organization for European Economic Co-operation* to establish "a supervision and emergency warning system in cases of increase in environmental radioactivity" is therefore an important and encouraging step in international collaboration in the field of nuclear energy. The main purpose of the supervision and emergency warning system is to facilitate and improve health protection measures in the event of a substantial increase of radioactivity in any member country of the Organization for European Economic Co-operation by immediately notifying it to the responsible authorities in the other member countries. The transmission of information is to be governed by three criteria: (1) health protection in the event of a nuclear incident; (2) health aspects of an increase in environmental radioactivity of undefined origin; and (3) scientific interest of any case of abnormal increase in environmental radioactivity. As monitoring stations for measuring airborne radioactive material are already in existence in all member countries of the Organization the proposed system is to be based at first on air monitoring, although the importance of water and dietary contamination is recognized, and extension of the system to these fields of monitoring is envisaged at a later stage.

The three levels of artificial total β-radioactivity in air, given as a basis for determining the categories of increase in radioactivity, are: (1) an 'emergency' level of the order of 1,000 μμc./m.^{3}; (2) a 'pre-emergency' level of the order of 100 μμc./m.^{3}; and (3) a level of 'scientific interest', set at ten times higher than the previous monthly average and exceeding the value of 5 μμc./m.^{3}. The levels are averages over one day, the 'emergency' level being determined in relation to possible nuclear incidents and regarded as valid for emergencies of short duration. Very approximately in terms of the Windscale reactor accident, the 'emergency' level corresponds to that observed within 50 miles (downwind) of Windscale itself, the 'pre-emergency' level to radioactivities observed at that time in Devon, and the level of 'scientific interest' to observations made at some places on continental Europe. It should be noted that the warning-levels are those of artificial β-radioactivity, and that average levels of natural β-activity in the air in Great Britain are typically of the order of 100 μμc./m.^{3}.

An administrative organization is laid down, and reports are to be made to the European Nuclear Energy Agency as well as to the "designated authorities" in the member countries. After an initial period of two years operation, the Steering Committee for Nuclear Energy is to make a report to the Council of the Organization for European Economic Co-operation on the way the system has worked.

Perhaps the most important aspect of the scheme is that it represents the beginning of collective action on a problem which transcends national boundaries, and that, as the Steering Committee itself suggests, "when the supervision and warning system is set up, the possibilities of more extensive international collaboration should be considered". There would appear to be no insuperable difficulties to complete international collaboration on this aspect of the peaceful uses of nuclear energy. F. W. SPIERS

* Report of the Steering Committee for Nuclear Energy and Council Decision on the Establishment of a Supervision and Emergency Warning System in Cases of Increase in Environmental Radioactivity. Organization for European Economic Co-operation. Paris. July 7, 1961.

MOLECULAR DISARRAY IN A CRYSTAL LATTICE PRODUCED BY A FISSION FRAGMENT

By DR. F. P. BOWDEN, C.B.E., F.R.S., and L. T. CHADDERTON
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IT should be possible, if we choose an appropriate crystal, to observe directly in the electron microscope the lattice disarray caused by the passage of a fission fragment, and an attempt has been made to do this. The first observations of crystal lattices and associated defects were made by Menter[1] with phthalocyanine and its metal derivatives. These are polycyclic hydrocarbons with large flat molecules and the shape of the molecule influences the mode of crystallization. Platinum phthalocyanine, for ex-

ample, crystallizes in the monoclinic form[2] as long flat needles and with a large unit cell ($a_0 = 23{\cdot}9$ Å., $b_0 = 3{\cdot}81$ Å., $c_0 = 16{\cdot}9$ Å., $\beta = 129{\cdot}6$ Å.). It is this property, in addition to the fact that the platinum atom, a heavy scatterer of electron waves, lies in the centre of the molecule, which facilitates the direct observation of (20$\bar{1}$) lattice planes, separated by 11·94 Å., in the electron microscope. In a carefully aligned electron microscope with a nominal resolving power of 'better than 10 Å.', transmission electron micrographs of thin unstrained crystals of platinum phthalocyanine possess parallel fringes the separation[3,4] and direction[5] of which correspond to (20$\bar{1}$) lattice planes in the crystal structure. The experimental detail so far is in full accord with the only extensive theoretical analyses published[6,7].

Platinum phthalocyanine was prepared from platinous chloride and phthalonitrile[8] and purified by repeated vacuum sublimation. Crystalline specimens suitable for the electron microscope examination were obtained in two ways. The first method involved simple mechanical grinding of the pure crystals, a drop of a suspension in ethyl alcohol being allowed to evaporate from a perforated carbon film on a copper electron microscope specimen grid. The second method, by far the more successful, consisted of condensing the crystals directly on to the support films from the vapour phase[3]. Crystals varying in thickness from some 40 Å. upwards could thus be prepared at will. Specimen grids were placed in contact with thin foils of natural uranium and irradiated with thermal neutrons in the experimental reactor *Bepo* at the Atomic Energy Research Establishment, Harwell, for periods of 5 min.–20 hr. at a pile factor of 12. After a few weeks the specimens irradiated with fission fragments and thermal neutrons were safe enough to handle and were examined in transmission at high magnification in a Siemens Elmiskop I electron microscope. Crystals which were not placed in contact with natural uranium foil, and which consequently suffered neutron irradiation only, were also examined.

In electron micrographs taken in transmission at low beam intensities with exposure times of up to 30 sec., the platinum phthalocyanine crystals show tracks and spots about 100 Å. in diameter. These can be attributed unambiguously to individual fission fragments, since they do not appear in crystals irradiated with thermal neutrons only and since their number correlates with that calculated from dose considerations. The tracks, which are sometimes dashed and which sometimes have an alternating light and dark contrast, would appear to result from fission fragments which are travelling at an oblique angle, or almost parallel, to the plane of the crystal. The spots, however, have a light contrast on a dark background and these may be attributed to fission fragments travelling at a high angle or perpendicular to the plane of the crystal. We shall consider here the damage due to particles such as these which pass normally through crystals about 100 Å. thick in a direction roughly parallel with the (20$\bar{1}$) planes.

Figs. 1 and 2 show what can happen to the (20$\bar{1}$) fringes when the damage is in the main body of the crystal. In Fig. 1, for example, the regular lattice is disordered and there is a roughly circular diffuse region which extends over about 80 Å. Close to the edge of the diffuse region the (20$\bar{1}$) fringes curve inwards towards the disordered region : this is shown in the adjoining diagram. In Fig. 2, however, the

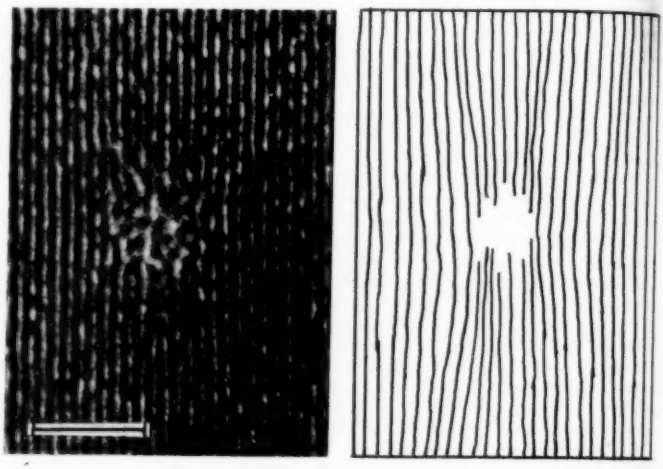

Fig. 1. Electron micrograph of a platinum phthalocyanine crystal showing the molecular disarray when a fission fragment travels perpendicular to the projection of the (20$\bar{1}$) planes and within the main body of the crystal. The drawing illustrates the situation more clearly. Scale, 100 Å.

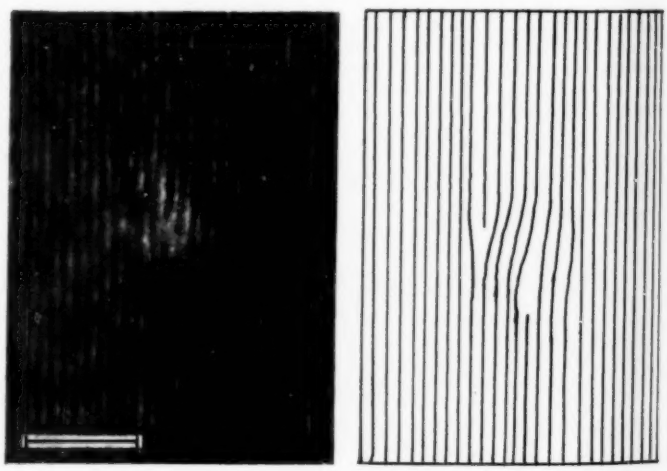

Fig. 2. Electron micrograph of a platinum phthalocyanine crystal showing damage sustained when a fission fragment travels perpendicular to the projection of the (20$\bar{1}$) planes and within the main body of the crystal. A dislocation dipole results. Scale, 100 Å.

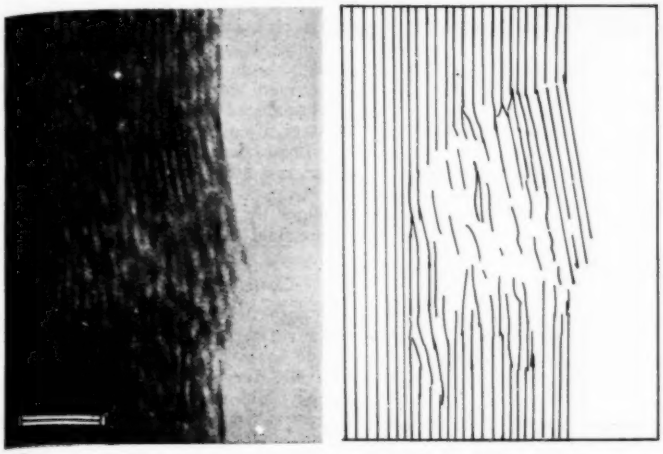

Fig. 3. Electron micrograph of a platinum phthalocyanine crystal showing damage sustained when a fission fragment strikes a crystal edge. Scale, 100 Å.

picture is quite different. Instead of a diffuse region the fringes are resolved clearly over the damaged area. Although those bordering this area are again curved, the reason for this is apparent; it will be seen that there are two fringes which terminate abruptly within the area of damage.

When the fission fragment penetrates the crystal close to one of its parallel edges, the damage is very much greater in extent. This is illustrated in Fig. 3.

If we assume that fringe patterns obtained with platinum phthalocyanine crystals in the electron microscope represent in some measure the behaviour of the (20$\bar{1}$) molecular planes (and for thin crystals the approximation is a good one[6]), then certain conclusions can be drawn from the above observations. In the first instance, a measure of the extent of the damage due to a fast-moving fission fragment is obtained. In the body of the bombarded crystal the diameter of the damaged region caused by one nuclear fragment varies between 80 and 120 Å. (that is, between 7 and 10 molecular planes). In Fig. 3, however, at a crystal edge, the appropriate figure is nearer 250 Å. The perfection of the (20$\bar{1}$) lattice planes is destroyed over this region and close to it there is evidence for a curvature of the molecular planes. An interesting conclusion may be derived from Fig. 2. It has been emphasized that a terminating half-fringe is not to be interpreted as a terminating half-plane, that is, an edge dislocation of Burgers vector (20$\bar{1}$)[10]. On the contrary, we can only infer that a dislocation of quite general character, with both screw and edge components, passes from the top to the bottom surface of the crystal. In Fig. 2, however, a Burgers circuit traced around the damaged region closes completely, and indeed we might have expected this since we started with a perfect lattice. It would seem that the effect of the fission fragment on the lattice has been the introduction of two dislocations of quite general character but of opposite polarity. The concept of the dislocation dipole is not entirely new—Bollman[11] has recently shown, using moiré fringe techniques, that dislocation dipoles may be present in neutron-irradiated graphite.

The energy of a fission fragment at birth is about 100 MeV. and the atomic weight may be anywhere between 72 and 161. Thus we are dealing with very fast moving particles ($\sim 10^9$ cm./sec.) and so the fragments have high charges ($\sim 18e$). It can be deduced, using a value of 1·70 eV. (Chadderton, L. T., unpublished work) for the low-energy limit of the main optical absorption band, that loss of energy by

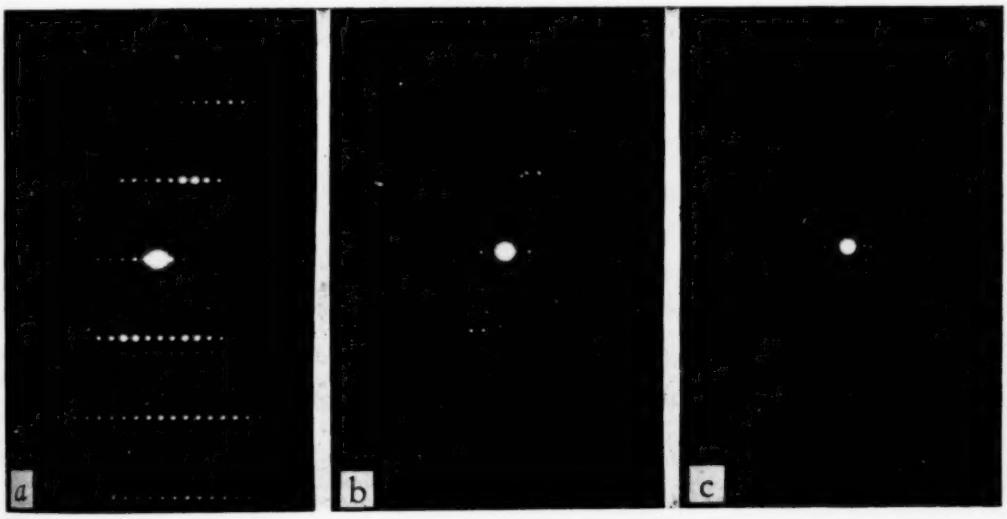

Fig. 4. Changes in the electron diffraction pattern from a thin single crystal of platinum phthalocyanine after irradiation with electrons at high magnification and low beam intensity: (*a*) after 40 sec.; (*b*) after 80 sec.; (*c*) after 120 sec. Apparently the platinum atoms remain roughly in their original positions

ionization[13] exceeds that due to elastic collisions by a large factor. The production of primary knock-ons by Rutherford collisions accounts for only about one part in a thousand of the total loss of energy. In such circumstances we may consider the resulting excitation to be a purely thermal temperature spike; the damage arises from the rapid delivery of heat to the lattice by the moving particle. Temperatures exceeding 1,000° C. endure for times in the neighbourhood of 10^{-11} sec., exact values depending on the thermal properties of the lattice. Since platinum phthalocyanine sublimes in air at temperatures above 500° C., it becomes evident that 'evaporation' will occur along the length of the cylindrical track of the moving fragment and that recrystallization, if it takes place, is unlikely to be in perfect register with the undamaged lattice. Bombardment of platinum phthalocyanine with heavy nuclear particles of high velocity and charge may therefore introduce dislocation arrangements into an initially perfect crystal. Other authors have examined the damage produced by fission fragments in mica[12-14], uranium oxide[15,16], and graphite[17], but without the assistance of direct lattice observations. Bowden and Montagu-Pollock have described the damage produced by a fission fragment in an explosive crystal[18].

Effect of Electron Bombardment

One important factor has been so far omitted, namely, the effect of the electron waves on the crystal while it is being examined and photographed in the electron microscope. The electrons themselves constitute a radiation capable of producing considerable damage to the lattice since they are accelerated to relativistic velocities by a high voltage of 80 kV. Their effect has been investigated with the assistance of the electron micro-diffraction device, whereby changes in crystal structure can be examined as irradiation with electrons proceeds. Fig. 4 shows what happens to the diffraction pattern from a single crystal of platinum phthalocyanine while it is being irradiated with electrons at a low beam intensity. A fringe pattern is obtainable from the crystal throughout the irradiation, but its nature and origin change. In the first instance the pattern is derived from the (20$\bar{1}$) molecular planes, but as the cumulative electron dose on the specimen builds up the crystal structure changes. All order in the lattice (except that which gives rise to the (20$\bar{1}$) and (201) spots in the diffraction pattern) disappears and the resulting fringe pattern observed in bright-field electron micrographs becomes much smoother. It would appear that although the large organo-metallic molecules are being destroyed, the platinum atoms remain roughly in their original positions in the (20$\bar{1}$) planes, embedded in a matrix of carbonaceous matter. The information derived from fission fragment bombarded crystals is, however, not affected by these considerations, since the electron micrographs were taken before decomposition by electron irradiation had proceeded to any marked extent.

Conclusion

We see that it is possible to make a direct observation of the molecular disarray produced in a crystal lattice when a single fission fragment passes through it. The extent of the damage can be measured to within one molecular plane and in platinum phthalocyanine varies between seven and twenty (20$\bar{1}$) planes, being dependent on where, and at what angle, the fragment strikes the crystal. In some cases dislocation dipoles are formed. The method appears to be a perceptive one for studying the detailed mechanisms of radiation damage.

One of us (L.T.C.) is indebted to the Department of Scientific and Industrial Research for the award of a research studentship.

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ELECTRON-SPIN RESONANCE SIGNALS FROM LYOPHILIZED BACTERIAL CELLS EXPOSED TO OXYGEN

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ESCHERICHIA COLI cells, lyophilized from distilled water, lose their viability very rapidly when exposed to oxygen, but not when kept in vacuum[1]. Certain aspects of these observations suggested that free radicals might be involved in this phenomenon. An investigation of electron-spin resonance was therefore undertaken. The details of the spectrometer have been described[2,3]. An associated analogue computer enabled the determination of the number of magnetic centres in the sample as compared with the diphenylpicrylhydrazyl (DPPH) standard[4].

Cells of *E. coli* strain *B/r* were grown on nutrient agar and, after collection, were washed and resuspended in distilled water. About 2×10^{11} cells were lyophilized in each ampoule. An electron-spin resonance sample tube (diameter 5 mm.) was sealed to each ampoule as a side arm. After completion of the lyophilization the samples were either sealed

* On leave of absence from the Israel Institute for Biological Research, Nes-Ziona, Israel, which is Dr. Lion's present address.

off under vacuum or filled with dried oxygen and then sealed. The dry bacterial powder was pulverized by some large-diameter glass beads which had been introduced into the ampoules before lyophilization. The powder was then poured into the side arm, which was introduced into the cavity of the spectrometer. Fig. 1 shows the spectra of two samples of dried cells. One had been exposed to oxygen for 2 days while the other remained in vacuum. The signal produced in presence of oxygen was apparent 30 min. after the beginning of exposure, when the earliest measurement was made, and reached its final height after some hours. After reaching a peak no change either in height or shape of the signal occurred for at least 4 months. Contrary to experiments performed on lyophilized tissue by Miyagawa *et al.*[5], the signal remained stable even after removal of oxygen by re-evacuation, as shown in Fig. 2. While heating the lyophilized cells to 100° C. for 75 min. after exposure to oxygen did not affect the signal obtained (Fig. 3, II), the cells heated at 100° C. for 5 min. before freeze-drying produced a much smaller signal after exposure to oxygen (Fig. 3, III).

This behaviour of free-radical signals present after lyophilization with respect to heat and exposure to oxygen differed from radiation-induced resonances. Dried bacteria irradiated with 750 kr. of X-rays showed a broad, weak, and double-peaked signal which became higher and sharper after heat treatment and rapidly decayed on exposure to oxygen (Fig. 4). This change in pattern after heating suggests a heat-induced transition from one radical species to another, and a fundamental difference between radiation-induced radicals and those occurring after lyophilization alone.

Fig. 1. First derivative of electron-spin resonance absorption curve of *E. coli* cells lyophilized from distilled water. I, 18 mgm. exposed to oxygen for 2 days: II, 21 mgm. kept in vacuum

Fig. 2. First derivative of electron-spin resonance absorption curve of *E. coli* cells lyophilized in distilled water and exposed to oxygen. I, before removal of oxygen by re-evacuation; II, after re-evacuation

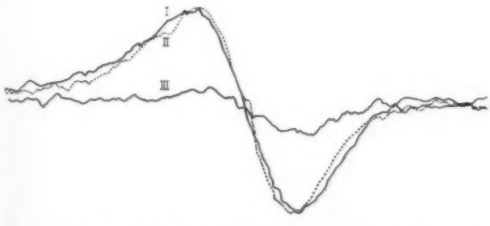

Fig. 3. First derivative of absorption curve of *E. coli* cells lyophilized from distilled water and exposed to oxygen. I, normal cells (60 mgm.): II, cells heated to 100° C. for 75 min. after exposure to oxygen (60 mgm.); III, cells heated to 100° C. for 5 min. before lyophilization (70 mgm.)

Dried *E. coli* cells may be protected against the lethal effect of oxygen by different substances, as, for example, by thiourea or even more effectively by sodium iodide[6], but cysteine (as hydrochloride partially neutralized to *p*H 6·0 with sodium hydroxide) is completely ineffective in this respect[6]. In a typical experiment the viability of dried cells after exposure to oxygen for 4 hr. at 28° C. was found to be: control, 0·05 per cent; in presence of cysteine, 0·001 per cent; in presence of thiourea, 42 per cent; in presence of sodium iodide, 80 per cent[6].

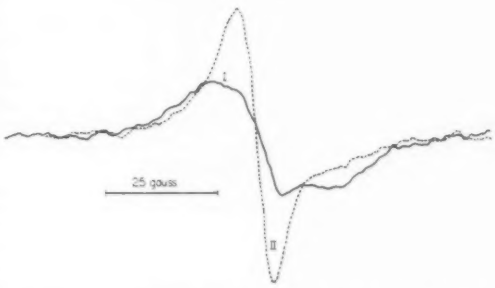

Fig. 4. First derivative of absorption curve of *E. coli* cells lyophilized and irradiated in vacuum by 750 kr. X-rays. I, before heating; II, after heating to 100° C. for 20 min. Spectrometer sensitivity about one-sixth that for other figures. All rates of scanning were equal

Measurements of electron-spin resonance were therefore made on dried cells exposed for 2 days to oxygen in the presence of these substances. The ampoules were then opened in relative humidity less than 35 per cent, and the fluffy material was rapidly compressed with a glass rod into sample tubes used in measuring electron-spin resonance, thus enabling greater amounts of material to be introduced into the sensitive volume of the cavity. The sample tubes were sealed immediately. Fig. 5 shows the signals produced when dried cells are exposed to oxygen in the presence of cysteine, thiourea, sodium iodide, and no additive. Each sample contained 50 mgm. of bacterial cells, the additives comprising a further 16–18 mgm. A qualitative relationship seems to exist between the ability of these additives to suppress the signal from electron-spin resonance of oxygen-exposed, dried bacterial cells, and their ability to protect these cells against the lethal effect of oxygen.

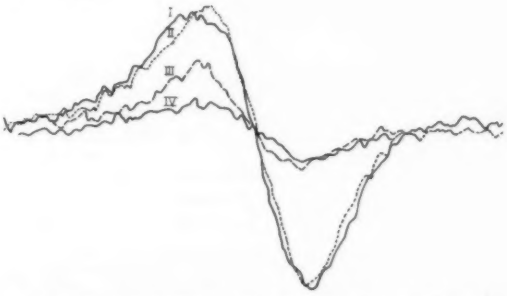

Fig. 5. First derivative of absorption curve of *E. coli* cells lyophilized and exposed to oxygen for 2 days in presence of I, cysteine; II, no additive (control); III, thiourea; IV, sodium iodide

The approximate number of magnetic centres produced by oxygen in dried bacteria is about 2×10^{16} spins/gm. This figure is probably correct only within a factor of two owing to the high gain needed in the detection system of the apparatus with consequent drift and noise when the signals of these materials were determined. Since the number of bacterial cells in 1 gm. of dry material is roughly 4×10^{12}, the number of free radicals produced for each dry cell reacting with oxygen is about 5,000. Possibly the oxygen reaction is quite specific and involves only few molecular species the normal state of which might be vital to the survival of the cell. It also seems that the 'oxygen acceptor' in this reaction is quite heat-sensitive while the reaction product is not.

The experiments of Miyagawa *et al.*[5] were done on ground, non-viable animal tissues and are therefore not directly comparable with our results. They show that molecular species of biological origin which in the dry state are able to react quickly with oxygen are not restricted to bacteria. Our results differ in that the reaction between oxygen and the sensitive site of bacteria seems to be irreversible.

This last observation would exclude a normal metabolic role for the reaction, for otherwise one might expect a resonance signal in dried bacteria (which are intact and viable cells) kept in vacuum. One might rather imagine that the site sensitive to oxygen is protected in a wet-cell system by the action of normal metabolic machinery in removing excess oxygen. Once this normal machinery is stopped in the dry cell, the site becomes easily accessible to oxygen. Unless other protecting materials are present, there follow consequent inactivation and loss of the capability of the cell to recover when it is re-introduced into its normal environment. This latter property of the bacterial cell makes it an interesting model for investigating biological phenomena by means of electron-spin resonance.

The Oak Ridge National Laboratory is operated by the Union Carbide Corporation for the U.S. Atomic Energy Commission.

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ANGULAR MOMENTUM OF CONTINUA

By Prof. J. S. DAHLER and Prof. L. E. SCRIVEN
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WHEN applied to the continuum model of matter, the principle of conservation of linear momentum becomes Cauchy's law of motion, which in spatial co-ordinates is the familiar equation of change of linear momentum. In Cartesian tensor notation, with D/Dt the substantial time derivative and other symbols having their usual meanings:

$$\rho \frac{DV_j}{Dt} = \rho F_j + T_{ij,i} \qquad (1)$$

The stress-tensor can be resolved into symmetrical and anti-symmetrical parts $T^{(s)}_{ij} + T^{(a)}_{ij}$; the second is given by $(1/2)\ e_{ijk}\ A_k$, where the pseudo-vector $A_k \equiv e_{kmn}\ T_{mn}$. With this resolution, the first moment of equation (1) about an arbitrarily chosen origin is found to be:

$$\rho \frac{D}{D_t}(e_{kij}\ R_i\ V_j) = \rho\ e_{kij}\ R_i\ F_j + (e_{kij}\ R_i\ T_{lj})_{,l} - A_k \qquad (2)$$

This equation of change of moment of momentum, or external angular momentum, $e_{kij}\ R_i\ V_j$ (per unit mass), shows that quantity to be conserved in the usual sense only when the stress-tensor happens to be symmetrical; for otherwise A_k does not vanish, and the last term represents local consumption (or production) of moment of momentum throughout the system. What is the fate of this external angular momentum?

A system may exchange with its surroundings not only linear but also angular momentum. The two classes of interaction need not be wholly equivalent in the case of real materials, although they are so regarded in most traditional continuum models. But just as the distant material surroundings may exert locally a vector body force F_j, they may also exert locally on the system a pseudovector body-couple G_j per unit mass: and just as the contiguous material surroundings may exert a vector surface traction $T_j = n_i\ T_{ij}$, they may also exert on the surface of the system a pseudovector couple $C_j = n_i\ C_{ij}$ per unit area, where C_{ij} is the couple stress-tensor. If the pseudovector L_k denotes the local density of angular momentum per unit mass, the principle of conservation of angular momentum for the constant mass system associated with an arbitrary, convected volume is:

$$\frac{d}{dt}\int_V \rho L_k dV = \int_V \rho(e_{kij}R_i F_j + G_k)dV + \int_S n_l(e_{kij}R_i T_{lj} + C_{lk})dS \qquad (3)$$

In the usual way this leads directly to the equation of change of total angular momentum:

$$\rho \frac{DL_k}{Dt} = \rho\, e_{kij}\ R_i\ F_j + \rho\ G_k + (e_{kij}\ R_i\ T_{lj})_{,l} + C_{lk,l} \qquad (4)$$

Even more important than this equation is the following, obtained by subtracting equation (2) from equation (4) and denoting as M_k the difference $L_k - e_{kij}\ R_i\ V_j$ between total angular momentum and moment of momentum:

$$\rho \frac{DM_k}{Dt} = \rho\ G_k + C_{lk,l} + A_k \qquad (5)$$

This equation of change of internal angular momentum makes clear the fate of the external angular momen-

tum: it is transmuted to the internal form by the alchemy of an unsymmetrical state of stress. After transformation it may be accumulated within the system or removed to the surroundings. Just as the body-force is the rate of arrival of linear momentum from distant material surroundings, the body-couple G_k is the rate of arrival of internal angular momentum from the same source; and in a similar analogy the net surface couple $C_{lk,j}$ is the rate of arrival, by diffusion, of internal angular momentum from contiguous material surroundings.

So far as we know, equation (5) was first given in complete form in 1959 by Dahler[1], who derived it as a general hydrodynamical equation in the context of a statistical mechanical theory of transport processes in fluids composed of polyatomic molecules. In his developments for classical and quantum cases, patterned after the methods of Irving and Kirkwood and of Irving and Zwanzig, a key step (as in the continuum development here) consists of distinguishing interactions of a system with distant from those with contiguous surroundings. Somewhat earlier Grad[2] had argued in favour of a version lacking only the body-couple term. Much earlier the Cosserats[3] had given another version lacking the accumulation term; long neglected, theirs has been revived in the modern theory of rheology (cf. refs. 4 and 5) and re-discovered in the theory of elasticity[6]. Other incomplete versions have appeared in various contexts, for example, the theories of liquid crystals and of what are called anisotropic fluids[7,8].

The derivation sketched here confirms that equation (5) is a basic law of continuum mechanics fully as general as Cauchy's. What are some of its consequences? In the first place, equations (2) and (5) indicate that moment of momentum and internal angular momentum are separately conserved only when the local state of stress is symmetrical. Therefore the usual proof of symmetry fails when there are couple stresses and accumulation of internal angular momentum, as Grad[2] has observed, as well as when there are body couples to be accounted for. This implies that whenever the adoption of a continuum model requires averaging over the centres of mass of sub-continuum units having rotational degrees of freedom, the stress-tensor is in general asymmetrical, its asymmetry stemming from the interaction of the centres-of-mass system with an interpenetrating twist or spin system[1]. An example is provided by Toupin's theory of the elastic dielectric[4]; a farther-reaching one is provided by the new Laval–LeCorre–Raman theory of elasticity[6,9,10]. Indeed, equation (5) is a means of joining that theory to extensive researches on nuclear and molecular spin systems in the solid state. It suggests, incidentally, a need for directing attention to the angular momentum aspect of magnetism in quantitative descriptions of macroscopic behaviour of not only solids but also fluids and less-well-defined forms of matter. As yet, in the physics of fluids there are few examples of antisymmetrical states of stress: predicted for polyatomic fluids in general by the statistical mechanical theory, they seem to have been first considered in detail only recently by Ericksen in a theory of certain non-Newtonian fluids[7,8]. The complete equation of internal angular momentum, which is basic to any full consideration of such states of stress, merits further attention in rheology, particularly as an aid to understanding non-linear behaviour. There might be interesting consequences for the hydrodynamic theory of helium II. The possible existence of asymmetrical stress seems to have been overlooked, and perhaps the peculiar system of spin-excitations in that liquid might be more conveniently described in terms of intrinsic angular momentum density and equation (5) than in terms of vorticity singularities. The equation does suggest the existence of unexplored effects of interchange between moment of momentum and internal angular momentum in macroscopic systems rotated at high angular velocities.

Another consequence of the angular momentum equation is the need it shows for discovering the constitutive relations giving the dependences of antisymmetrical stress, couple stress, and body-couples on a set of independent variables that completely describes the dynamical state. This need compels one to recognize that continuum models of real matter must be endowed with point structure, a notion that goes back to Duhem and the Cosserats[3–5]. This structure must then be characterized quantitatively. Further work on energy and entropy accounting, on constitutive relations, on certain applications to polar fluids, and on consequents for turbulent flow is in progress.

We are grateful to Prof. W. H. Schwartz, of Stanford University, for bringing the most recent papers by Ericksen to our attention.

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APPLICATION OF PALÆOMAGNETISM TO GEOLOGICAL CORRELATION

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THE Monteregian Hills are formed from the erosional remnants of eight igneous plugs which intrude the Palæozoic sedimentary beds of south-eastern Quebec (Fig. 1). Work on these intrusive rocks[1] by a number of geologists has shown that they were derived from a common magmatic source of basic composition.

Suggestions have been made[2] to include in the Monteregian Hills series a number of alkaline intrusives which are located outside the immediate area

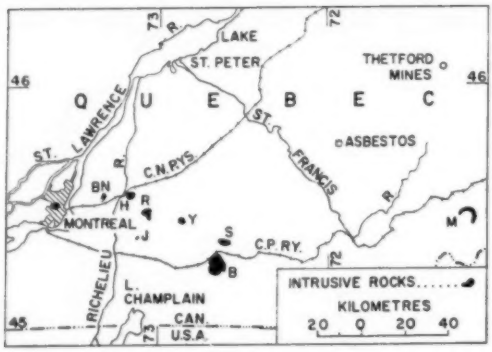

Fig. 1. Areal distribution of Monteregian hills (*BN, H, R, J, Y, S, B*) and Mount Megantic (*M*)

of the Hills. One of these bodies forms the collar of basic rocks on the outer rim of Mount Megantic, some seventy-five miles east of the main group. This body (labelled *M* in Fig. 1) was recently shown (Reid, A. M., unpublished M.Sc. thesis, University of Western Ontario, 1961) to bear petrological affinities with the Monteregian Hills series. The contemporaneity of the Mount Megantic rocks and the Monteregian Hills is suggested in this article on the basis of palæomagnetic data.

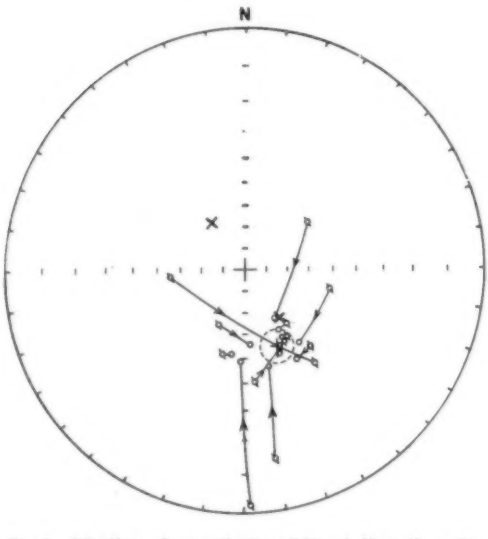

Fig. 2. Directions of magnetization of Mount Megantic rocks. ϕ, original readings; ×, mean direction for other Monteregian hills; ○, after magnetic cleaning; ⊕, circle of confidence and mean direction for Mount Megantic rocks

A suite of twelve oriented specimens was collected from different sections of the basic part of Mount Megantic. For the sampling, a solar compass was used to eliminate the possible error that could be introduced by the interaction of the magnetic needle of an ordinary compass with the relatively strongly magnetic rocks dealt with. Two 1-in. cubes were cut from each specimen and their magnetization directions were measured. The mean direction of magnetization of the two cubes was taken as representative of the specimen. For the purpose of eliminating unstable components of magnetization in the rocks, the latter were then submitted to the action of a decreasing amplitude, alternating field in a magnetic-field-free space. The maximum initial field amplitude used was 250 œrsteds. This treatment improved the clustering of the magnetization directions a considerable amount, as illustrated in Fig. 2.

The mean direction of magnetization for the group is represented by a cross centring the dotted line circle of confidence, the radius of which was computed using Fisher's method.

The fact that the parameter $\hat{K}$ of the group is relatively high, coupled with the fact that the mean direction of magnetization is almost diametrically opposed to the direction of the present field of Earth at the collecting site, suggest strongly that the mean direction given in Fig. 1 is that of a stable component of magnetization in the rocks and that it reflects the attitude of the ambient magnetic field of Earth at the time the rocks cooled from magmatic temperatures.

In a previous study[2], I found that the mean directions of magnetization of the Monteregian Hills lie along an axis represented by two *X*'s in Fig. 2. The attitude of this axis was computed from the mean directions of magnetization of two groups of rocks which were respectively polarized downward in the north-west quadrant and upward in the south-east quadrant. Although the magnetization direction of the Mount Megantic rocks is almost parallel to this axis, it is more accurate, for the sake of geological correlation, to compare the pole positions, which may be inferred from the Mount Megantic and the Monteregian Hills palæomagnetic directions respectively. The reason for this is the remoteness of the two groups of collecting sites. These pole positions are represented stereographically in Fig. 3, and they are labelled '*m*' and '*M*' respectively. Their proximity and the overlapping of their respective ellipse of confidence strongly suggest that the Mount Megantic

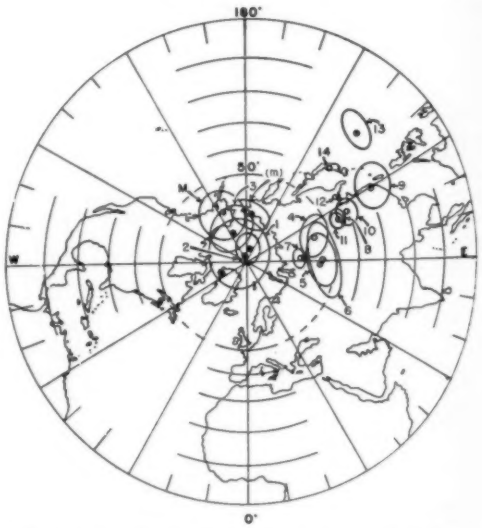

Fig. 3. Palæomagnetic pole positions inferred from North American rocks. *M*, Monteregian Hills, Mount Megantic excluded: *m*, Mount Megantic; 1, Tertiary rocks; 2, Pliocene and Miocene rocks; 3, Cretaceous rocks; 4, 5, 6, 7, Triassic rocks; 8, 9, 10, Permian rocks; 11, 12, Carboniferous rocks; 13, Silurian rocks

Table 1. Directions of Magnetization of Basic Intrusive Rocks
N, Number of samples; θ, radii of circles of confidence; $\hat{K}$, dispersion parameter; D, declination; I, inclination

	N	θ	$\hat{K}$	D	I
Mount Megantic	12	6·5	44	157	−52
Other Monteregian Hills	10	18°	7·9	340	+57
Other Monteregian Hills	39	9°	7·5	142	−62

Table 2. Palæo Pole Positions

	Latitude	Longitude
Monteregian Hills	65° N.	157° W.
Mount Megantic	68·5 N.	171·5 E.
Dakota sandstones*	74 N.	168 W.

* Pole position as computed by me from directions of magnetization and collecting sites given by Runcorn (1956).

Table 3

	Mineral in parent rock	Age in 10^6 yr.	Laboratory
Mount Royal	Pyroxene in Tinguaite	100	M.I.T.
Mount Johnson	Biotite in Essexite	110	M.I.T.
Mount Brome	Biotite in Nordmarkite	122	G.S.C.
Mount Megantic	Biotite in Gabbro	115	G.S.C.

Potassium/argon age determinations by Geological Survey of Canada (G.S.C.) and the Massachusetts Institute of Technology (M.I.T.).

basic rocks and the Monteregian Hills were intruded at the same time.

Pole positions derived from other North American rocks of known ages[4] are also plotted in Fig. 3. It appears from these data that a marked shift of the pole occurred between Cretaceous and Triassic times. It would seem, therefore, that pre- and post-Triassic rocks could be distinguished on the basis of their palæomagnetic directions. If this line of reasoning were legitimate, the 'M' and 'm' poles belong to the post-Triassic group and their proximity to the Cretaceous pole would suggest further a Cretaceous age for both the Mount Megantic and the Monteregian Hills rocks. This conjecture was recently supported by potassium/argon age determination data (Table 3) released by two independent laboratories on samples collected from three of the Monteregian Hills and from Mount Megantic. Thus, palæomagnetic and radiogenic data support independently the synchronous intrusions of these two rock units and place them in Cretaceous times.

I thank the Director of the Geological Survey of Canada for permission to publish this work.

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DIFFERENTIAL SECONDARY MAGNETIZATION IN SOME BRITISH CAMBRIAN ROCKS

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TWELVE orientated rock specimens were collected from three horizons in the Hartshill Quartzites and the Hyolithus Limestones from two localities near Nuneaton. Geologically[1] the quartzites include true quartzites and ferruginous sandstones coloured red or pink, while the limestones consist of organic material in a fine-grained matrix of calcite, iron minerals and other material, the whole strongly red stained. The rocks dip to the south-west at about 40 deg.

Magnetic results were obtained for eleven rocks and are plotted on the equal area stereonet (Fig. 1) in their field orientations; the twelfth was too weak to give significant results with the existing magnetometer. Combining the vectors for the four specimens from the Hyolithus Limestones by taking the mean of the direction cosines and making a statistical estimate of the dispersion after Fisher[2] gives:

$$D\ 017°\quad I\ 23°\quad \alpha\ 16°\quad \varkappa\ 33$$

The following abbreviations are used: D, declination measured from true north; I, inclination to the horizontal, positive downwards; α, semi-angle of the cone of confidence drawn at the 95 per cent probability-level; $\varkappa$, estimate of precision. In contrast, the mean direction obtained from the seven specimens from the quartzites is:

$$D\ 132°\quad I\ 64°\quad \alpha\ 25°\quad \varkappa\ 6$$

Correcting for the dip of the rocks moves both the mean directions towards the south-west by about 20 deg., so that when these directions are compared with those found in Cambrian rocks from Wales[3] the quartzites give a similar direction, but the mean vector for the limestones is about 90 deg. removed.

All the rocks were demagnetized in a.c. fields of up to 100 œrsteds using apparatus similar to that described by Creer[4]. The new vectors obtained on re-

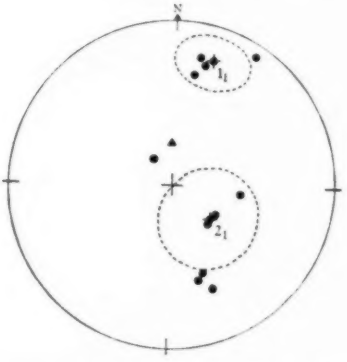

Fig. 1. Equal area stereonet showing the positions of the magnetic vectors for eleven rocks from the British Cambrian together with the group mean vectors. All vectors are in field orientation. ●, Vector plotting in lower hemisphere; 1_1, mean vector for limestones; 1_2, mean vector for limestones after a.c. washing; 2_1, mean vector for quartzites; 2_2, mean vector for quartzites after a.c. washing; 2_3, mean vector for quartzites after a.c. washing and correction for dip; ○, vector for Trias rocks; ■, vector for Cambrian rocks; ▲, direction of present dipole field. The broken lines show the circles of confidence drawn at the 95 per cent level

measuring (Fig. 2) give mean directions for the quartzites:

$$D\ 161^\circ \quad I\ 55^\circ \quad \alpha\ 15^\circ \quad \times 14$$

and for the limestones:

$$D\ 015^\circ \quad I\ 44^\circ \quad \alpha\ 9^\circ \quad \times 115$$

where the rocks are in their field orientations. Both groups show a reduction in the amount of scatter and both show a displacement of the direction of the mean towards the south-west which cannot be attributed to the removal of a component of secondary magnetization parallel with the present geomagnetic field.

The displacement of both mean vectors in a similar direction would also rule out the possibility that the limestones were originally magnetized in a reverse direction to the quartzites and have both taken on a component of secondary magnetization to give their present positions. The presence of a reversal is also unlikely since the limestones occur stratigraphically within the quartzite series, samples from above and below the limestone horizon give similar directions of magnetization and there is no evidence of any marked break in deposition.

Comparing the change in the intensities of magnetization produced by the a.c. washing there is a marked difference between the limestones, which lose about 85 per cent, and the quartzites, which show little change in intensity. This suggests that the two rock types have taken on very different components of secondary magnetization.

The mean vectors for the two groups in field orientation are given in Fig. 3 both before and after a.c. washing together with mean vectors for rocks of Trias age from Europe[5,6] and for the Caerbwdy Sandstones of Cambrian age from Wales[3]. The mean direction for the Hyolithus Limestones before washing lies within the group of Trias results and accords well with those from rocks of Bunter age; the effect of the washing is to shift the mean direction towards the Cambrian direction. For the Hartshill Quartzites the mean before washing lies close to the Cambrian direction, and the washing moves the vector still closer so that if this mean direction is corrected for the observed dip of the rocks the resultant vector:

$$D\ 193^\circ \quad I\ 34^\circ \quad \alpha\ 16^\circ \quad \times 12$$

is in good accord with the Cambrian direction.

Combining the magnetic and geological evidence these observations may be explained as follows: The Hartshill Quartzites and the Hyolithus Limestones were weakly magnetized parallel to the geomagnetic field in Cambrian times. Uplift and erosion having removed any overlying rocks, these Cambrian rocks lay at or near the surface in the Trias. During the deposition of the thick series of red beds of Trias age which are now exposed to the north-west of the Cambrian rocks, these were penetrated by iron-bearing ground waters which, in depositing new iron minerals and oxidizing existing minerals to new forms, imposed a chemical remanent magnetization[7,8]. The degree to which the Cambrian rocks were affected by this process depended on their permeability; this was slight in the quartzites, but greater in the more permeable limestones. That this new magnetization of the limestones could not be removed completely by washing in a.c. fields up to 100 œrsteds is in agreement with Creer's work[4] on unstable Keuper Marls, which in some cases still retained components of secondary magnetization after washing in fields of up to 850 œrsteds.

If it is correct to assume that the quartzites after a.c. washing give a true record of the direction of the Cambrian geomagnetic field the position of the magnetic pole at that time can be calculated as:

$$\text{Lat. } 18^\circ \text{ N.} \quad \text{Long. } 165^\circ \text{ E.} \quad d\psi\ 10^\circ \quad d\chi\ 18^\circ$$

where $d\psi$ and $d\chi$ are the lengths of the semi-axes of the elipses of confidence drawn at the 95 per cent probability-level. This pole position is in good agreement with those determined from the British Cambrian and from rocks of similar age from Norway[9].

This work was carried out while I was a student at the Sedgwick Museum, Cambridge, and I take this opportunity to thank W. B. Harland and Dr. R. Hey of that department for their help and encouragement.

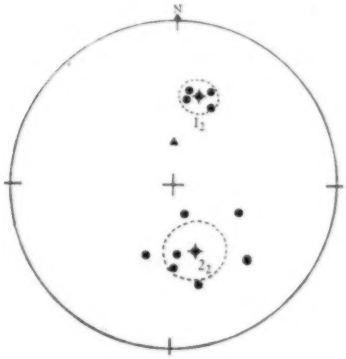

Fig. 2. Equal area stereonet showing the positions of the magnetic vectors after a.c. washing, together with the group mean vectors. All vectors in field orientation. Symbols as in Fig. 1

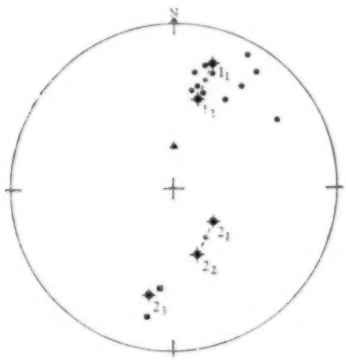

Fig. 3. Equal area stereonet showing the relation of the mean positions for these Cambrian rocks to directions determined by other workers from Trias and Cambrian rocks. Symbols as in Fig. 1

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AUTO-IMMUNIZATION AND LIVER CIRRHOSIS

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RECENT progress of work on auto-immunization is remarkable, and the occurrence of this unusual condition has been reported or suggested in various sorts of diseases, one of which is liver cirrhosis. Gajdusek[1] applied the complement-fixation reaction widely and revealed the existence of auto-antibodies against the liver as well as several other tissue homogenates in cases of some chronic diseases, including liver cirrhosis. Tsujino[2] succeeded in producing by means of an immunological method some experimental condition which he called cirrhosis-like liver. Recently, King[3] reported a case of liver cirrhosis in which many *LE*-cells were revealed, and with a review of similar conditions reported, he concluded that some kinds of liver cirrhosis should be a manifestation of auto-immune reaction of the liver.

In our recent work, we tried to reveal the presence of an auto-antibody in serum against liver tissue obtained from the same individual, which we thought to be the real auto-immunization. As shown in Table 1, fifteen cases have been tested so far, most of which at autopsy revealed various kinds of liver alterations such as cirrhosis or fibrosis, liver cell necrosis, primary liver cell carcinoma, etc. Age of the patients varied between 8 and 74 yr., and all these were Japanese males. The time of autopsy after death varied from 2·25–15 hr.; most of them were rather fresh and less than 4 hr., during which period the bodies were not kept under refrigeration. In one case of primary liver cell carcinoma, both the tumorous and non-tumorous parts of the liver were examined. As for the cases with metastatic tumours in the liver, special care was taken to avoid contamination of the liver specimen with the tumour tissue.

A small piece (20–30 gm.) of the liver tissue and a cup of blood were collected from each case of autopsy. Within 24 hr., the blood was centrifuged, and the separated serum was incubated in a water bath kept at 56° C., for 30 min. Sodium azide was added in 0·1 per cent as a preservative. On the other hand, the tissue specimen was weighed and cut with a razor blade into small pieces; four times by weight of saline solution was added. This crude suspension was homogenized thoroughly, first with a Waring blender, and next with tissue grinder. After sodium azide (0·1 per cent) was added the tissue homogenate was centrifuged (5,000 r.p.m., 20 min.) and the supernatant was preserved for the serological test. A small segment of tissue was put into formalin solution for histological study.

To reveal auto-antibodies, Ouchterlony's agar reaction method[4], modified by Wilson and Pringle[5], was used. Interactions between matched serum and liver tissue were chiefly observed.

Among fifteen cases examined, two showed a positive reaction, as shown in Table 1. Incubation for 4 days at 38° C. was necessary to develop a definite line on the agar plate in one case (No. 2), and it took 10 days in the other (No. 14). Table 1 also shows the histological feature and weight of the liver of each case. None of the five cases, including one each of hæmolytic anæmia and chronic nephritis,

Table 1. Results of Autopsy Examination.

Case No.	Age (yr.)	Sex	Autopsy (hr. after death)	Pathological diagnosis (brief histopathological findings of the liver)	Liver weight (gm.)	Serological reaction
1	74	M	15	Stomach carcinoma (marked, periportal fibrosis and moderate congestion)	720	(−)
2	60	M	2½	Œsophagus carcinoma (fine-nodular cirrhosis and three nodes of metastasis)	1,540	(+)
3	71	M	3	Acquired hæmolytic anæmia (extra-medullary hæmatopoiesis in periportal areas and sinusoids)	940	(−)
4	19	M	2½	Stenosis of inferior vena cava (irregular fibrosis and granular pseudolobuli formation)	1,950	(−)
5	72	M	2¾	Stomach carcinoma (meso-nodular, atrophic cirrhosis with necrosis and tumour metastasis)		(−)
6	45	M	3¾	Iatrogenic massive liver necrosis (extensive, total necrosis and fatty degeneration of liver cells)	985	(−)
7	14	M	2¾	Primary liver cell carcinoma (hepatocellular carcinoma in non-cirrhotic, icteric liver)	2,260	(−)
8	68	M	5½	Stomach carcinoma (multiple, nodular metastases in congestive, atrophic liver)	1,390	(−)
9	30	M	3½	Chronic glomerulonephritis (cloudy swelling of liver cells, marked)	1,680	(−)
10	8	M	6½	Acute bulbar paralysis and poliomyelopathia (slight congestion of liver)	625	(−)
11	51	M	2½	Accessory sinus carcinoma (two, small nodular-metastases and slight lymphocytic infiltration)	1,404	(−)
12	70	M	2½	Hæmochromatosis (hæmochromatotic liver cirrhosis with degenerative change)	900	(−)
13	68	M	3	Aortic aneurysma, liver cirrhosis (macro-nodular cirrhosis with marked, nodular hyperplasia)	1,040	(−)
14	36	M	6	Fatty cirrhosis of the liver (fine-nodular cirrhosis with marked, fatty metamorphosis)	1,500	(+)
15	37	M	5½	Liver cirrhosis (fine-nodular, atrophic, irregular cirrhosis with scarring)	780	(−)

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which showed no remarkable primary liver change at autopsy, gave positive results in the serological test. Actually, there were ten cases with more or less altered liver tested in our work, among which none of the cases of acute liver necrosis, primary liver cell carcinoma, and liver fibrosis showed the development of auto-immunization. A total of six cases of cirrhosis of the liver were tested, and both the cases which showed serologically positive results were in this last group.

Grossly, no atrophy was noticed in either of those two cirrhotic livers which showed positive serological reaction; one weighed 1,540 gm. and the other 1,500 gm. Three metastatic tumour nodes were found in the liver of case No. 2, but they were too small to influence the weight of the liver.

Microscopical investigation of those cases revealed an interesting fact that the two were quite similar also in histological character. Sections of the liver in those two cases showed rather small, uniform pseudolobules surrounded with thin trabeculæ of fibrous tissue, without any marked scar formation. Lymphocytic infiltration and bile-duct proliferation in portal areas were seen, but quite slight. Neither nodular hyperplasia nor adenomatous growth of liver cells was noticed. Elastosis was not marked. Fatty metamorphosis of the liver cells was especially marked in case No. 14. As shown in Table 1, the cirrhotic or fibrotic livers tested in this work showed various histological features, and the only two cases of those which had quite the same gross and histological characters gave a positive auto-immune reaction. This fact was considered to be too serious to be neglected as a matter of accidental coincidence. Moreover, those two cases had one common feature in regard to previous histories, namely, alcoholism. The liver of those two cases showed the feature of mild, slowly-progressing cirrhosis of a rather portal type which could be classified as nutritional when both clinical and pathological results were considered.

The definite serological interactions were found only in cases of liver cirrhosis so far as examined, and they could be exclusive for certain types of this disease. Even so, the reason why the auto-antibodies against the liver tissues were found only in cases of a so-called nutritional type of cirrhosis are still unknown. However, the fact that numerous fat droplets were revealed histologically in the lung capillaries of those cases apparently suggests the possibility of an outflow of the fat extracts of the liver cell components into the blood stream in the case of severe fatty metamorphosis of the liver, which might cause developing auto-immunization.

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COUNTERACTION BY CYSTEINE OF GLYCOGENOLYSIS AND INHIBITION OF MICROSOMAL PROTEIN SYNTHESIS PRODUCED BY DIMETHYLNITROSAMINE

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WITHIN a few hours of administration of dimethylnitrosamine (DMNA) and before any other lesion can be observed, there is found a severe inhibition of amino-acid incorporation into the proteins of the isolated microsomal-soluble fraction of rat liver[1,2]. Indirect evidence has been presented[1] that DMNA is not inhibitory in itself, but that the compound is first converted by an oxygen- and reduced triphosphopyridine nucleotide-dependent *N*-demethylase, located in the microsomal membranes, to the *N*-hydroxymethyl (methylol) derivative. The latter is very reactive and unstable so that either this derivative, or the formaldehyde released therefrom, may act as the toxic agent *in situ*. Moreover, the monomethylol derivative might also give rise to diazomethane[2]. Any one of these derivatives may react with susceptible groups of the enzymes, cofactors or ribonucleic acid (RNA) templates of the protein synthetic process[1]. Although it is not known which of the possible derivatives, $O{=}N{-}N{=}(CH_2OH)_2$, H_2CO or CH_2N_2, is in fact responsible for the toxic action of DMNA, all three may react with sulphydryl groups which have been recognized[3] as cofactors of protein synthesis at the microsomal level. It is of interest that the lesion, in incorporation of amino-acid produced by DMNA, is located in the microsomes[1,2] and not in the soluble (*s*) fraction which catalyses amino-acid activation and the transfer of the latter to *s*–RNA. Next to its effect on protein synthesis, DMNA has also been found to cause the disappearance of glycogen from rat liver[4]. Three hours after the administration of DMNA (50 mgm./kgm. body-weight), when changes in the endoplasmic reticulum[4] and an inhibition of microsomal protein synthesis were observed, the glycogen content appeared, both by electron microscopy and chemical analysis, to be normal, whereas 17 hr. later almost all the glycogen had disappeared. Three hours after a two-fold greater dose of DMNA, 20 per cent (average) of the glycogen had disappeared, the bulk of the latter being lost in the period 3–10 hr. after DMNA injection.

In the present experiments we have been able to show that administration of cysteine to rats markedly counteracts both the percentage inhibition of microsomal protein synthesis and the glycogenolysis which result from the injection of DMNA. Cysteine administration according to the régime listed in the legend of Table 1 (or slightly varied) caused a marked increase in the *in vitro* protein labelling of the later isolated microsomal-soluble fraction of liver by leucine-1-^{14}C (50–150 per cent increase in 8 experiments). A relatively greater effect (150–350 per cent increase) of cysteine administration was always

Table 1. *In vitro* INCORPORATION OF LEUCINE-1-^{14}C INTO THE PROTEINS OF THE MICROSOMAL-SOLUBLE FRACTION AND THE GLYCOGEN CONTENT OF LIVERS FROM NORMAL AND DMNA-TREATED RATS AS AFFECTED BY CYSTEINE ADMINISTRATION

DMNA	Cysteine	Protein radioactivity of microsomal-soluble fraction		Glycogen content of liver slices
		(counts/min. at 'infinite' thickness)	(per cent change)	(per cent of wet weight of liver)
—	—	685		8·1
—	+	1409	+106	6·1
+	—	201	−71	2·2
+	+	832	+314 −41	5·5

150 mgm. DL-cysteine was administered subcutaneously twice daily for 2 days to 300-gm. rats of the inbred strain *U*. Early next morning the rats received saline (0·1 ml.) or DMNA (100 mgm./kgm. body-wt.) dissolved in saline intravenously and another 150 mgm. of cysteine subcutaneously. These, and similarly treated rats which had not received the cysteine, were killed 5 hr. later. The incorporation of leucine-1-^{14}C into the proteins of the isolated microsomal-soluble fractions (10,000*g* supernatant) was studied as described in ref. 1. Glycogen was measured in slices prepared from the same livers by the anthrone method.

observed on the decreased incorporation of amino-acid of the corresponding fractions prepared from the livers of rats 3–10 hr. after injection of DMNA. The experimental results in Table 1 illustrate a case in which the rats had been subjected to the toxic effect of DMNA for 5 hr. Experiments of this kind can only be interpreted in terms of an increase in amino-acid incorporation, or of a counteraction by cysteine of the DMNA-inhibited amino-acid incorporation, if the radioactivity of the immediate precursor is known. If cysteine in some way or another had produced a decrease in the concentration of the endogenous leucine of the soluble fraction, a smaller dilution of the added ^{14}C-leucine and an increase in labelling of protein would have resulted. The following results, however, make it likely that this was not the case.

Experiments on the incorporation of amino-acid into the proteins of liver slices of the various rats, treated according to Table 1, gave results identical to those of Table 1. Since the increase in the incorporation of amino-acid into the proteins of the liver slices of rats which had received cysteine (controls: 40 and 66 per cent, DMNA-treated: 62 and 112 per cent increase in two experiments) was not accompanied by an increase in carbon-14 content of the respiratory carbon dioxide derived from leucine-1-^{14}C, as compared with the corresponding data obtained with rats which had not received cysteine, it followed that the increase in protein labelling was not due to a decrease in the amount of endogenous leucine. The results of four experiments in which the 105,000*g* supernatants of the livers from four rats, treated according to Table 1, were incubated with leucine-1-^{14}C and adenosine triphosphate, followed by isolation (phenol method) and purification (through 'Sephadex') of the ^{14}C-leucyl-*s*-RNA formed, supported the latter conclusion. The radioactivity of the control- and DMNA-treated preparations (± cysteine) was identical. This suggests that both the stimulatory effect of cysteine on the incorporation of amino-acid system of the normal rats and the counteraction by cysteine of the percentage inhibition by DMNA is exerted at the microsomal level.

It is shown in Table 1 that cysteine administration also counteracted the glycogenolysis produced by DMNA, some measure of variation in this and the above effect of cysteine being noted with individual rats. Cysteine administration (150 mgm. twice daily for 2 days) also resulted in, or markedly prolonged, the survival of rats which had received a lethal dose of DMNA (22·5 mgm./300 gm. rat, given immediately after the fourth cysteine administration; all controls dying within 40 hr.).

It may thus appear that cysteine, although not being tolerated too well by some of the rats (especially the younger ones), and apart from its more specific effect on microsomal protein synthesis (compare the controls), prevents or counteracts DMNA toxicity in general. It is of interest that cysteine and cysteamine (cysteamine *per se* shows also some measure of toxicity) also protect against a lethal dose of nitrogen mustard[5]. Although not permitting a definite choice, the correspondence in protective effect of cysteine against DMNA and the alkylating agent nitrogen mustard, might indicate that the methylating agent diazomethane is the actual toxic derivative of DMNA. The protective effect of cysteine against DMNA toxicity may be due to a direct interaction of the sulphydryl groups of the former with the highly reactive and toxic DMNA derivative, or to a transitory blockage of tissue SH-groups which are thus unable to react with the DMNA derivative.

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DETECTION OF A POSSIBLE INTERMEDIATE IN OXIDATIVE PHOSPHORYLATION

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A STRIKING feature of oxidative phosphorylation is that although occurrence of the process has been recognized for some twenty years, no phosphorylated intermediates have been demonstrated. The formation of three or more molecules of adenosine triphosphate (ATP) for each reduced nicotinamide or flavin-containing co-enzyme oxidized by oxygen suggests participation of one or more intermediates. Several investigations[1–3] have indicated that mitochondria, in the absence of an added phosphoryl acceptor, may accumulate material capable of allowing rapid formation of ATP. Whether the observations reflect the presence of a phosphorylated intermediate or some other form of energy storage is uncertain. Data on ADP ⇌ ATP exchange have been interpreted as indicating a phosphoenzyme intermediate[4]. Various findings, in particular the demonstration that oxygen between the β- and

γ-phosphorus atoms of ATP is furnished by ADP[5], strongly infer that any phosphorylated intermediates formed are derived from orthophosphate (P_i), followed by reaction with ADP to give ATP. Present information does not exclude, however, the possibility that the first covalent compound formed from both P_i and ADP in one or more steps of oxidative phosphorylation is ATP itself.

The importance of detection and isolation of any intermediates in oxidative phosphorylation to the understanding of the reaction mechanism served as the motivation for our work. As reported in preliminary communications[6], these investigations have led to the demonstration of the rapid uptake of $^{32}P_i$ into a protein-bound form. Conditions affecting the labelling of this unidentified phosphate suggest its participation in oxidative phosphorylation, and presentation of some details in this regard, together with some properties of the substance, is the principal purpose of this article.

Rapid Formation of an Unidentified Phosphate

Conditions which might be expected to favour accumulation of an intermediate of oxidative phosphorylation in mitochondria are incubation in the presence of excess substrate, oxygen and P_i, but without added ADP. In our experiments, mitochondria have been incubated under such conditions but in the absence of added P_i, then exposed for short periods to a trace of high specific activity $^{32}P_i$. The trace of added $^{32}P_i$ to the medium was avoided because such addition would reduce the possibility of detecting a small fraction of the total added phosphorus-32 that might be present in any intermediate. Stopping of the reactions and nearly complete removal of $^{32}P_i$ and AT^{32}P from the protein-bound phosphorus-32 was achieved by adding a concentrated urea solution containing a non-ionic detergent to give apparent solubilization of the mitochondria[7], followed by passage through a column of anion exchange resin. After incubation for a few sec. or more with $^{32}P_i$ presence of ^{32}P in this unidentified material and in AT^{32}P are shown in Fig. 1. In this and other comparable experiments, the average amount of ^{32}P in the unidentified fraction has been slightly greater at 10 sec. than at 1 min. The tendency for the decrease in 1 min. sometimes noted might reflect decrease in specific activity of $^{32}P_i$ resulting from interchange with P_i and ATP pools of mitochondria. Evidence exists for the presence of intramitochondrial P_i which does not readily mix with medium P_i (refs. 10, 5).

The rate of labelling of the unidentified phosphate is much more rapid than that of phospho-proteins or phospho-lipids, as reported by Conover *et al.*[11] and by Burnett and Kennedy[12], and thus does not appear to represent the initial uptake into a large pool of phosphorylated compounds. A time-course as given in Fig. 1 is in harmony with participation of the unidentified phosphate as an intermediate. A rate of labelling comparable with or slower than that of ATP would not, however, preclude participation of a compound as an intermediate in view of possible effects of relative reaction-rates as well as compartmentation in mitochondria. An effect of relative reaction-rates arises from the demonstrated dynamic reversal of ATP formation accompanying oxidative phosphorylation[13,14]. The pathway of phosphorus-32 may be simply represented as follows:

$$P_i \rightleftharpoons \text{phosphorylated intermediate} \rightleftharpoons \text{ATP}$$

After addition of $^{32}P_i$, the specific activity of the intermediate would be expected to be similar to that of the P_i if the interchange in the first step were rapid compared with that in the second step. Conversely, the specific activity of the intermediate would approximate that of the ATP if the interchange in the second step were more rapid. Indeed, in some of our experiments with small variations in conditions, the rate of appearance of ^{32}P in ATP and in the unidentified phosphate has been similar.

The absolute amount of phosphorus-32 appearing in the unidentified fraction is only about 0·2–0·3 per cent of the added $^{32}P_i$. It became important, therefore, to assess the possibility that the small activity found might arise from experimental artefact and not represent a metabolically derived phosphate compound. Controls in which $^{32}P_i$ or AT^{32}P was added with or after the concentrated urea demonstrated that appearance of the product depends on incubation for the short periods with mitochondria. That the product very likely is a derivative of P_i is shown by the liberation on short heat treatment at neutral *p*H of the radioactivity in a form extractable with added P_i by isobutanol-benzene from acid molybdate[8] and precipitable with P_i as magnesium ammonium phosphate. In addition, the product had a radioactivity with penetration characteristics and half-life similar to ^{32}P.

The experiments have been hampered considerably by the presence of varying amounts of impurities in $^{32}P_i$ as received which appear in the column eluates of controls. Of various purification techniques, that used at present, and which is successful with most samples, is as follows: The sample is made alkaline with ammonia, adsorbed on to a small column of 'Dow-1-bicarbonate' anion exchange resin, and the

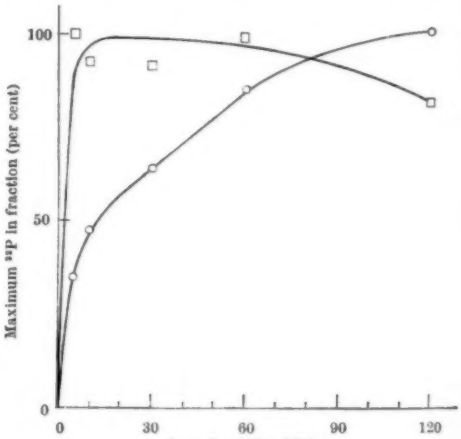


Fig. 1. Time-course of appearance of $^{32}P_i$ in ATP and in the unidentified phosphate. A mixture containing 11 m*M* sodium glutamate, 6 m*M* KCl, 240 m*M* sucrose, 1 m*M* ethylenediamine tetra-acetate (EDTA) and mitochondria from roughly one-fourth of a rat liver in a total volume of 2 ml. was incubated with rapid stirring under oxygen at *p*H near 7 and 15° C. for 1 min. A drop of high specific activity $^{32}P_i$ was then added, and the reaction stopped at the desired time by additions of 6 vol. of cold 6 *M* urea and 4 per cent detergent ('Triton X-100'). The sample was passed through a column (about 0·8 × 8 cm.) of 'Dow-1-Cl' and ^{32}P in the eluate measured. The incorporation of $^{32}P_i$ into ATP was estimated as the ^{32}P not extracted by isobutanolbenzene in the Berenblum and Chain assay (ref. 8), performed on the supernatant solution after precipitation of protein by cold 0·3 *M* perchloric acid. The urea–'Triton' and the sucrose solutions were purified by passage through a mixed ion-exchange resin. The mitochondria were prepared essentially as described by Schneider and Hogeboom (ref. 9) with 1 m*M* EDTA, *p*H 7·4, added to the 0·25 *M* sucrose

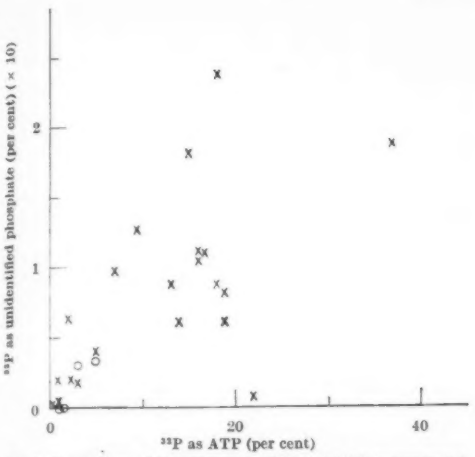


Fig. 2. Comparison of the percentage of the total $^{32}P_i$ incorporated into the unidentified phosphate and ATP. Experimental conditions were as given in Fig. 1, with incubation for 30 sec. after $^{32}P_i$ addition, for results designated by crosses. For results designated by circles, 10^{-3} *M* 2,4-dinitrophenol was present

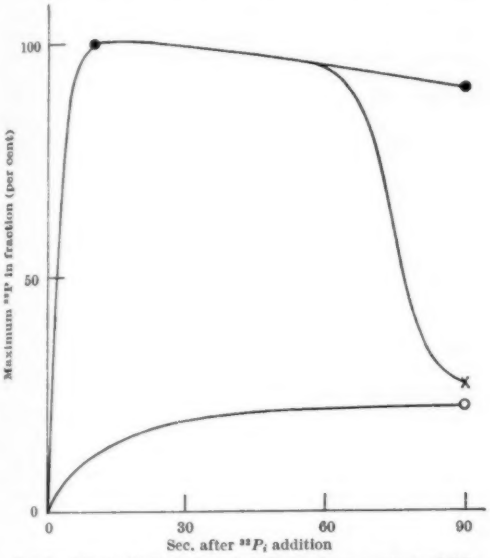


Fig. 3. Effect of added P_i and ATP on the percentage of $^{32}P_i$ appearing in the unidentified phosphate. Experimental conditions were as given in Fig. 1, with addition of P_i and ATP to give final concentrations of 1 m*M* each at the times indicated. ●, No added P_i and ATP; ×, P_i and ATP added at 60 sec.; ○, P_i and ATP added before $^{32}P_i$

$^{32}P_i$ eluted with 0·15 *M* ammonium bicarbonate–0·013 *M* ammonia. Mixing of the column eluate with 'Dow-50(H^+)' removes ammonia and liberates carbon dioxide to give a $^{32}P_i$ solution low in total salt concentration. This procedure has given $^{32}P_i$ in which controls showed less than 1 per cent as much phosphorus-32 in column eluates as did incubated samples.

The amount of the unidentified phosphate detected, although representing only a small fraction of the $^{32}P_i$ added, is about comparable on a molar basis with the level of cytochromes present, as estimated from the data of Chance and Williams[1]. If one assumes that the specific activity of the unidentified phosphate is equal to that of the P_i of mitochondria, the maximum amount detected is roughly 0·05 μmole per gm. of rat liver mitochondrial protein. The possibility exists that this amount represents only partial phosphorylation of the precursor of the unidentified phosphate.

Production of material from a given preparation of mitochondria is fairly reproducible, but different preparations show considerable variation in their ability to incorporate $^{32}P_i$ into both the unidentified phosphate and ATP. A summary of results collected over a period of several months with liver mitochondria is given in Fig. 2. These show that mitochondrial preparations with ability to accumulate a higher per cent of the added trace amount of $^{32}P_i$ as ATP show a tendency for formation of more of the unidentified substance. On storage for a few hours at 0°, ability to form both intramitochondrial AT^{32}P and the unidentified substance declines.

A similar labelling of the unidentified phosphate is obtained with glutamate, α-ketoglutarate, β-hydroxybutyrate or succinate as substrates or without added substrate. The material has also been detected in mitochondria from rat liver and heart, as well as ox liver and heart and rabbit heart.

Effect of Added P_i and ATP and of Inhibitors on the Unidentified Phosphate

Any substance which was an intermediate in the formation of ATP from P_i would be expected to undergo rapid interchange with added P_i or ATP or both. The results given in Fig. 3 show that addition of unlabelled P_i and ATP after incorporation of ^{32}P into the unidentified phosphate readily results in loss of ^{32}P from the fraction. Clearly, a dynamic interchange between the fraction and P_i–ATP exists. Further, addition of unlabelled P_i and ATP before

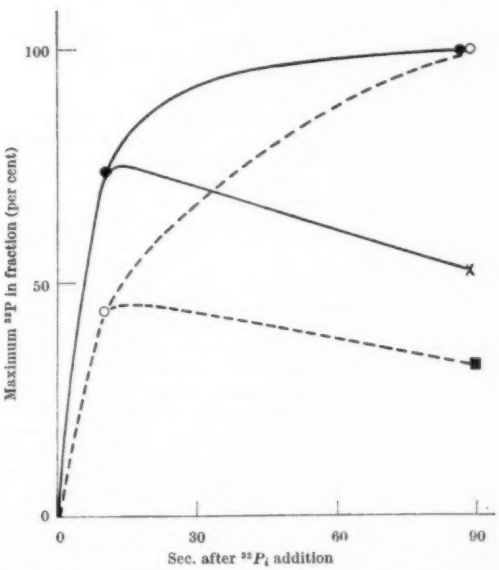


Fig. 4. Effect of addition of 2,4-dinitrophenol on the labelling of the unidentified phosphate and ATP. Experimental conditions were as noted with Fig. 1, except that the added urea was at *p*H about 9·5 and no detergent was present. 2,4-Dinitrophenol additions were made as noted. The solid lines indicate the ^{32}P in the unidentified phosphate and the dashed lines the ^{32}P in the ATP. ●, ○, No DNP; ■, ×, 10^{-3} *M* DNP at 10 sec.

Table 1. Representative Effects of Inhibitors on Labelling of Unidentified Phosphate and ATP

Exp.	Inhibitor	Sec. after $^{32}P_i$ addn.	Percentage added as $^{32}P_i$ as Unidentified phosphate	ATP
1	None	10	0·07	9
	2,4-Dinitrophenol, 10^{-4} *M*	10	0·06	6
	2,4-Dinitrophenol, 10^{-3} *M*	10	0·01	0
	None	30	0·10	14
	2,4-Dinitrophenol, 10^{-4} *M*	30	0·04	5
	2,4-Dinitrophenol, 10^{-3} *M*	30	0·00	1
	None	90	0·14	34
	2,4-Dinitrophenol, 10^{-4} *M*	90	0·04	11
	2,4-Dinitrophenol, 10^{-3} *M*	90	0·02	3
2	None	30	0·11	20
	Cd^{++}, 10^{-5} *M*	30	0·02	1
3	None	30	0·14	27
	Oligomycin, 12 μgm./ml.	30	0·13	6
4	None	30	0·07	30
	Oligomycin, 50 μgm./ml.	30	0·06	9
	2,4-Dinitrophenol, 10^{-3} *M*	30	0·02	5
5	None	30	0·08	18
	2,4-Dinitrophenol, 10^{-4} *M* + Oligomycin, 100 μgm./ml.	30	0·01	2
6	None	30	0·17	6
	KCN, 10^{-3} *M*	30	0·05	3
	Amytal, 3×10^{-3} *M*	30	0·04	4
	p-Mercuribenzoate, 10^{-3} *M*	30	0·00	0

Conditions were essentially as given with Fig. 1 with rat liver mitochondria unless otherwise specified. In some experiments, urea solutions made alkaline with NH_3 were used instead of urea-detergent solutions. 2,4-Dinitrophenol was added 1 min. before $^{32}P_i$. Cd^{++} were added 5 min. before $^{32}P_i$. Oligomycin, KCN, amytal and *p*-mercuribenzoate were present during 5–10 min. at 0° and 1 min. at 15° before $^{32}P_i$ addition. In experiment 6, ox liver mitochondria were used. Mitochondria from about 1 gm. of liver were used in experiments 1, 3, 4 and 6, and from about 0·2 gm. of liver in experiments 2 and 5.

the trace of $^{32}P_i$ nearly prevents incorporation of phosphorus-32 into the unidentified phosphate. This is the expected result from marked diminution of the specific activity of $^{32}P_i$.

The effect of various inhibitors of oxidative phosphorylation establishes a close relationship between the labelling of the unidentified phosphate and oxidative phosphorylation. Presence of 2,4-dinitrophenol, a well-recognized 'uncoupler' of oxidative phosphorylation, both reduced the formation and accelerated the disappearance of the fraction, as shown by the results in Fig. 4 and Table 1. The effectiveness of a given concentration of 2,4-dinitrophenol was not as great as might at first be anticipated from its known action on net oxidative phosphorylation and on the P_i–ATP exchange reaction; 10^{-4} *M* 2,4-dinitrophenol nearly completely abolishes these processes[12,15]. As noted from Table 1, considerable phosphorus-32 was incorporated into the unidentified phosphate even in the initial presence of 10^{-3} *M* dinitrophenol. Two factors may be responsible for this. First, with decrease in the concentration of mitochondria, lower concentrations of 2,4-dinitrophenol are more effective; and secondly, the inability of 2,4-dinitrophenol, when initially present, sharply to reduce the labelling of the unidentified phosphate was paralleled by continued ability to label intramitochondrial ATP. This could reflect a resistance of intra-mitochondrial ATP to breakdown in presence of 2,4-dinitrophenol as noted by Azzone and Ernster[16].

Effects of other inhibitors are also summarized in Table 1. Of particular interest are the effects of oligomycin. This antibiotic has been shown by Lardy, Johnson and McMurray[17] to prevent ATP formation without an 'uncoupling' action. Uptake of $^{32}P_i$ into the unidentified phosphate fraction is not affected or actually slightly increased when mitochondria are exposed to oligomycin. Under these conditions, formation of AT^{32}P is markedly decreased. This gives strong evidence that the unidentified phosphate ^{32}P arises from $^{32}P_i$ and not from AT^{32}P. Further, as shown in Table 1, presence of 2,4-dinitrophenol decreases formation of the unidentified phosphate in presence of oligomycin.

Cadmium ions, which Jacobs and Jacobs have shown to be potent inhibitors of oxidative phosphorylation[18], also prevent formation of the unidentified phosphate. Azide, which has effects like both 2,4-dinitrophenol and oligomycin[19], as well as an inhibitory action on electron transport, also is effective in reducing formation of the unidentified phosphate. Cyanide and presence of a nitrogen atmosphere cause some, but not marked, decreases in incorporation of $^{32}P_i$ into the unidentified phosphate. These effects of inhibitors on formation of the unidentified phosphate are similar to the effects noted on the $^{32}P_i$–ATP exchange reaction[13,16].

Properties and Fractionation of the Unidentified Phosphate

The unidentified phosphate as present in column eluates is non-dialysable, retained on charcoal, and is separable from P_i by passage through 'Sephadex *G*-50'. It is partly cleaved to P_i but mostly precipitated by brief exposure to dialysed eluates to cold 0·3 *M* trichloracetic acid, and is precipitated by acidic or neutral ammonium sulphate, ammonium sulphate plus dodecyl sulphate or ethanol. It is not extracted from lyophilized fractions by ethanol, 3 to 1 ethanol : diethyl ether, or acetone. Treatment of the unidentified material with a bacterial proteinase has yielded a radioactive product which is dialysable and not precipitated as magnesium ammonium phosphate. Dialysis of column eluates in presence of crude alkaline phosphatase markedly accelerates the appearance of $^{32}P_i$ in the dialysing medium. These various findings suggest that the phosphoryl group of the unidentified phosphate is attached to a co-factor firmly bound to the protein or lipo-protein of mitochondria or to an amino-acid side-chain.

After heating the dialysed eluates in a boiling water bath for 5 min. at neutral *p*H, the phosphorus-32 readily passes through charcoal and is identifiable as P_i. A *p*H-stability curve as based on the charcoal absorption test is shown in Fig. 5. The half-life at *p*H 7 and 77° is 22 min. First-order rate plots for the decomposition at *p*H 7 are essentially linear, giving strong indication that only one phosphorus-32 compound is present. The rate of decomposition is not markedly modified by the presence of hydrogen

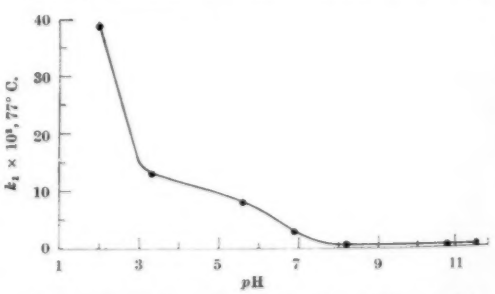
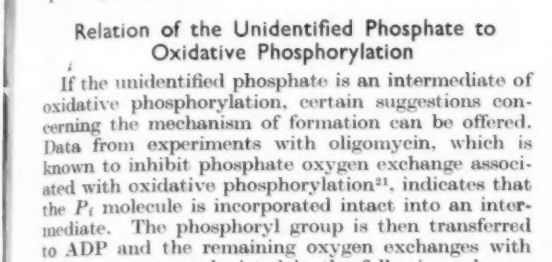


Fig. 5. Effect of *p*H on the stability of bound $^{32}P_i$. The first-order rate constant, k_1, was determined as follows: the eluates from columns after extensive dialysis against 0·02 *M* P_i, *p*H 6·8, were used directly in cleavage measurements. The *p*H was varied by addition of HCl or NaOH, the sample heated at 77°, cooled, and the *p*H adjusted to near 7. The amount of bound fraction remaining was approximated by the amount retained on charcoal; $^{32}P_i$ appears in the eluate

peroxide, hydrosulphite, molybdate, 2,4-dinitrophenol or *p*-mercuribenzoate, by ionic strength variation, or by replacement of air by nitrogen, but is about doubled in the presence of bromine. The data are in agreement with attachment to a nitrogen, sulphur or oxygen atom in a structure not susceptible to hydroxyl ion attack but modifiable by bromine. The stability work appears to eliminate the possibility of attachment of the phosphoryl residue to a seryl side-chain. The unidentified phosphate is considerably more stable and shows a different dependence of stability on *p*H than 2,3-dimethyl-1,4-naphthaquinol 1-phosphate[20]. Ubiquinone monophosphate is, however, more stable than the methyl substituted naphthaquinol phosphate[20].

Relation of the Unidentified Phosphate to Oxidative Phosphorylation

If the unidentified phosphate is an intermediate of oxidative phosphorylation, certain suggestions concerning the mechanism of formation can be offered. Data from experiments with oligomycin, which is known to inhibit phosphate oxygen exchange associated with oxidative phosphorylation[21], indicates that the P_i molecule is incorporated intact into an intermediate. The phosphoryl group is then transferred to ADP and the remaining oxygen exchanges with water oxygens as depicted in the following scheme:



followed by a rapid oxygen exchange,

$$Y - OH + H^{18}OH \rightleftharpoons Y - {}^{18}OH + HOH$$

Reversal of the above reactions would give rise to an exchange of P_i oxygens. Such a possibility has been suggested by Boyer *et al.*[5,13] and by Drysdale and Cohn[22]. Furthermore, even though the search for possible phosphorylated intermediates is by no means complete, the finding at this time of only one apparent candidate for an intermediate together with other data suggests consideration of mechanisms by which the various oxidation steps are in some manner coupled to formation of a common phosphorylated precursor to ATP.

In summary, the conditions governing the formation and disappearance of the unidentified phosphate fraction are consistent with, but by no means prove that, the substance is an intermediate in oxidative phosphorylation.

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MECHANISM OF ACTION OF LIVER ALCOHOL DEHYDROGENASE

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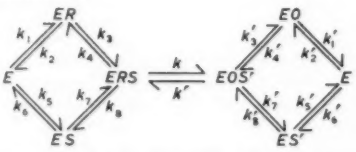
SEVERAL lines of inquiry have now shown that liver alcohol dehydrogenase (LADH) acts by forming binary and ternary complexes with coenzymes and substrates[1]:



There are two reaction phases with interconversion between them. (LADH and YADH represent liver and yeast alcohol dehydrogenase, respectively. *E* represents 1/2 LADH, since each molecule has two independent binding sites. *R* and *O* represent reduced (DPNH) and oxidized (DPN) diphosphopyridine nucleotide, respectively. *S* and *S′* represent aldehyde and alcohol, respectively.) It has also been confirmed that this complicated mechanism can, from a kinetic point of view, be described by the Theorell–Chance mechanism[2]:

$$E + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ER$$

$$ER + S \underset{k_3'}{\overset{k_3}{\rightleftharpoons}} EO + S'$$

$$EO \underset{k_1'}{\overset{k_2'}{\rightleftharpoons}} E + O$$

However, among previous attempts at experimental confirmation[3–5], there had been indications that at lower *p*H the ternary complexes affected the rate[4,5].

Kinetics without Inhibitors

For the general two-substrate equation:

$$e/v = \varphi_0 + \varphi_1/[S_1] + \varphi_2/[S_2] + \varphi_{12}/[S_1][S_2]$$

(primed symbols represent the reverse reaction), Dalziel[6] has derived the relations $\varphi_0 = \varphi_0'\varphi_2'/\varphi_{12}'$, $\varphi_0' = \varphi_1\varphi_2/\varphi_{12}$ and $K_{eq.}$ $(0{\cdot}9 \times 10^{-11}\ M)$ (ref. 7) = $\varphi_{12}/\varphi_{12}' = \varphi_0\varphi_1\varphi_2/\varphi_0'\varphi_1'\varphi_2'$, which distinguish the Theorell–Chance mechanism from other possible alternatives. Consequently, Lineweaver–Burk plots were determined for various concentrations of each constant partner and the intercepts and slopes plotted against these concentrations to give the desired φ-values[6]. Initial rates were measured on a sensitive fluorimeter with pen recorder[8], by following the DPNH fluorescence as DPN does not fluoresce. Table 1 shows the experimental φ-values. The relationship between them fulfils very well the sensitive requirements of the Theorell–Chance mechanism. The rate constants calculated on the basis of that mechanism and the binary dissociation constants, $K_{E,ald.}$, $K_{E,alc.}$, $K_{E,R}$ and $K_{E,O}$, calculated from the rate constants are also given. The agreement of the binary enzyme–coenzyme dissociation constants $K_{E,R}$ and $K_{E,O}$, with the values determined spectrophotofluorimetrically in equilibrium experiments in the absence of substrates (below), underlines further the operation of the Theorell–Chance mechanism. There are several reasons for the simplifications in the overall mechanism which lead to Theorell–Chance kinetics:

Table 1. 0·1μ Phosphate, 23·5° C., *p*H 7

(*a*) φ-Values and Theorell–Chance mechanism rate constants (1/φ).

φ_0 = 0·0135 sec.	k_2' = 74 sec.$^{-1}$
φ_1 = 0·090 sec. μM	k_1 = 11·1 sec.$^{-1}$ μM^{-1}
φ_2 = 3·2 sec. μM	k_2 = 0·31 sec.$^{-1}$ μM^{-1}
φ_{12} = 0·9 sec. μM (ref. 2)	
φ_0' = 0·32 sec.	k_2 = 3·12 sec.$^{-1}$
φ_1' = 1·9 sec. μM	k_1' = 0·525 sec.$^{-1}$ μM^{-1}
φ_2' = 82 sec. μM	k_2' = 0·012 sec.$^{-1}$ μM^{-1}
φ_{12}' = 10·500 sec. μM (ref. 2)	

(*b*) Calculated equilibrium constants for binary complexes.

$K_{E,R}(k_2/k_1)$ = 0·28 μM	$K_{E,O}(k_2'/k_1')$ = 141 μM
$K_{E,alc}(k_2'/k_2')$ = 1060 μM	$K_{E,ald}(k_2/k_2)$ = 10·0 μM

(1) $k_1 \approx k_7$ and $k_1' \approx k_7'$. The rate constants for the binding of the coenzyme are nearly independent whether the enzyme is free or already combined with the substrate; (2) k and k' are both high, the ternary complexes being in rapid equilibrium; (3) $k_4 \gg k_3$ and $k_4' \gg k_3'$. The ternary complexes prefer to liberate the substrates first, these being less tightly bound than the coenzymes. Thus, the dissociation velocity of the binary complexes in the last phase of the reaction becomes rate-limiting when both substrate and coenzyme are present in high concentration in the first phase and the maximum velocities are k_2 and k_2' rather than k_6 and k_6'. k_1 and k_1' determined in the presence of high concentrations of aldehyde or alcohol really reflect the 'on' velocity constants k_7 and k'_7, which, since the equilibrium and kinetic values of $K_{E,R}$ and $K_{E,O}$ are practically the same, means that $k_1 \approx k_7$ and $k_1' \approx k_7'$.

Spectrophotofluorimetric Dissociation Constant Determinations

Previously, fatty acids were found to form *EI* and *EOI*, but no *ERI* complexes, while the amides formed

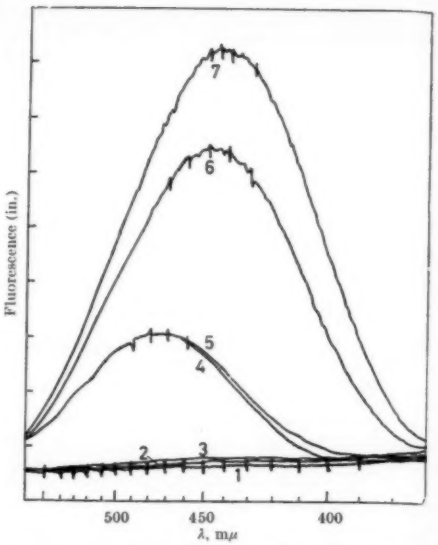


Fig. 1. Fluorescence emission curves for LADH, DPNH and imidazole. Excitation = 330 mμ
(1) Buffer (0·1μ phosphate, *p*H 7, 23·5° C.); (2) buffer + 0·1 *M* imidazole; (3) buffer + 0·1 *M* imidazole + 2·2 μM LADH; (4) buffer + 2·1 μM DPNH; (5) buffer + 2·1 μM DPNH + 0·1 *M* imidazole; (6) buffer + 2·1 μM DPNH + 2·2 μM LADH (*ER*); (7) buffer + 2·1 μM DPNH + 2·2 μM LADH + 0·1 *M* imidazole (*ERI*)

EI and *ERI*, but no *EOI* complexes[9]. Many nitrogen compounds have now been found to form ternary complexes with both *R* and *O*, and imidazole has proved particularly interesting, as it forms complexes with zinc ions[10] and LADH contains zinc[3] like YADH[11], while the ternary imidazole complex *ERI* also had an even greater fluorescence than *R* and *ER* (Fig. 1). This increased fluorescence may be due to interaction between the pyridine ring and zinc, although greater rigidity[12,13] and most probably energy transfer[14] have also been suggested. The formation of the *ER* and *ERI* complexes is also shown by the different maximum wave-lengths of their emission spectra.

Table 2 summarizes the dissociation constants determined using the recording spectrophotofluorimeter (a Bausch and Lomb grating monochrometer to isolate the activating wave-lengths was coupled to a Beckman *DU* spectrophotometer with pen recorder to analyse the emitted fluorescence[8]). Except for $K_{E,O}$ and $K_{EO,I}$ the dissociation constants are essentially invariant with *p*H. However, $K_{E,R}$ increases rapidly above *p*H 9 (ref. 5), suggesting that SH-groups join the protein to the DPNH pyridine ring[15]. The $K_{E,O}$ values were now higher than before, which resulted in the disappearance of previous disagreements between the equilibrium and kinetic values at lower *p*H (ref. 5), the results now agreeing throughout with the Theorell–Chance mechanism. They also show that with LADH–DPNH, the fluorescent and active sites are identical, which is apparently not the case with glutamic dehydrogenase[16]. It is noticeable that at *p*H 6, *ER* is a thousand-fold more stable than *EO*, at *p*H 9 only twenty-fold, the change being caused largely by the increase in the stability of *EO*. The variation of $K_{E,O}$ with *p*H was found to fit a monovalent dissociation curve which indicated the presence of a titratable group with a *pK* of 8·6

Table 2. DISSOCIATION CONSTANTS FOR LADH, COENZYME AND IMIDAZOLE COMPLEXES. μ = 0·1, 23·5° C.

(The values are calculated using the concentration of un-ionized imidazole)

pH	$K_{E,R}$ μM	$K_{ER,I}$ mM	$K_{E,I}$ mM	$K_{EI,R}$ μM	$K_{E,O}$ μM	$K_{EO,I}$ mM	$K_{EI,O}$ μM
6	0·23	4·2	0·47	2·01	266	0·80	448
7	0·31	3·5	0·55	2·03	160	1·45	430
8	0·41	3·6	0·68	2·20	51	5·9	440
9	0·65	3·0	0·67	2·91	12	24·2	435

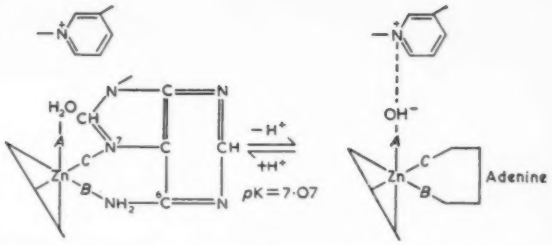
in the free enzyme, and of 7·1 in the binary *EO* (DPN–LADH) complex.

Preliminary experiments with YADH indicated complex formation similar to that with LADH, while lactic dehydrogenase gave no such indication, which supports the hypothesis that imidazole and certain nitrogen compounds are joined to zinc and lactic-dehydrogenase does not contain zinc[17].

Kinetics with Imidazole and Inhibitors

Whereas with fatty acids and amides the ternary complexes were more stable than the binary[9], the opposite is the case with imidazole (Table 2). The influence on the kinetics of caprate, *iso*butyramide (IB) and imidazole was therefore studied. For the general mechanism described here, $k_1 \approx k_7$ and $k_1' \approx k_7'$. This was found to be an example of a general rule that partners in a ternary complex do not seem to interfere appreciably with each other in regard to 'on' velocity constants, but mainly with the 'off' velocity constants, these increasing or decreasing to the same extent as there is mutual repulsion or stabilization in the complex. While caprate and IB had only inhibitory actions, imidazole was interesting as at high concentrations of substrate and coenzyme it gave a stimulation of reaction velocity due to the formation of labile ternary complexes in the second phase of the reaction (Fig. 2), but, when either concentration was low enough, inhibition occurred.

Imidazole, which forms both the ternary complexes *ERI* and *EOI*, also competed with both aldehyde and alcohol. This behaviour of imidazole, in view of its complexing properties with zinc, strongly suggests that zinc binds the substrates. That the binding of both coenzymes is weakened or disrupted by imidazole favours the idea that the adenine moiety which, unlike the pyridine ring, is the same in the coenzymes, forms a bidentate chelate to zinc[16]. Inhibition experiments with the bidentate ligands orthophenanthroline and $\alpha\alpha'$-dipyridyl[18], and equilibrium experiments with glycine, all of which do not form ternary complexes and are strictly competitive with the coenzymes, as would be expected when only three bonds are free and the coenzyme–adenine forms a bidentate itself, support this. They also indicate that the zinc bonds are octahedral[20], that zinc plays a central part in the binding of coenzymes and substrates[16,21], and that it is bound by three bonds to the protein. In the natural β-isomer of DPNH the pyridine ring is so close to the adenine that energy transfer takes place between them[22], and, since adenine is presumably bound to zinc, the pyridine ring must also lie close to zinc. In the free enzyme the free zinc bonds presumably hold water molecules, one of which at high *p*H (*p*H 8·6) loses a proton, the resulting attraction between $ZnOH^-$ and the positively charged pyridinium ring of DPN accounting for the increase in the stability of the DPN–LADH complex with increasing *p*H.



This does not operate with DPNH–LADH, where the pyridine ring carries no charge, while introduction of imidazole instead of OH^- eliminates the attraction effect, as is observed (Table 2).

Zinc is also implicated as the centre of the ternary complexes[16]; one bond which, in *ER*, accommodates a water molecule and in *EO* is joined to the pyridine ring, now is occupied by the substrate. Caprate was found to compete with DPNH and alcohol, *iso*butyramide with aldehyde and DPN, and imidazole, with the exception of DPNH, with all four partners. This suggests that alcohol and caprate have a different site of attachment from aldehyde and *iso*butyramide with imidazole able to go on either site. Thus, while the *A*-bond of the DPN complex would be the bond at which alcohol is attached, it would not seem to be the bond for aldehyde. However, two possibilities arise: (*a*) *Two bonds*: here a type of three-centre problem arises with one of the adenine bonds switching or flicking over. Thus, alcohol would be on bond *A* and DPN across *B* and *C*. During the rapid interconversion *EO*,alc. ⇌ *ER*,ald. the adenine-N(7) would remain on bond *C* and the adenine-NH_2 would switch to the *A*-bond, DPNH thus being across *AC* and aldehyde on the *B*-bond (or the N(7) could flick to *A*, *C* becoming the aldehyde bond). Thus with DPN on two of the zinc bonds, *B* and *C*, the *A*-bond will only

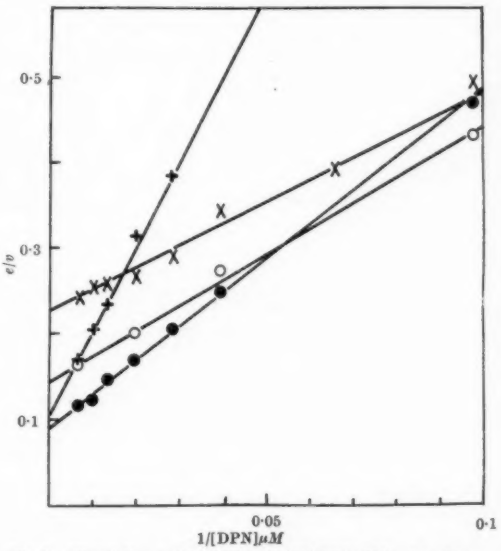


Fig. 2. Stimulation and inhibition by imidazole (0·1μ phosphate, 23·5° C. and *p*H 9)
×, No imidazole; ○, 2 mM; ●, 10 mM; +, 50 mM; [alc] = 6·1 mM

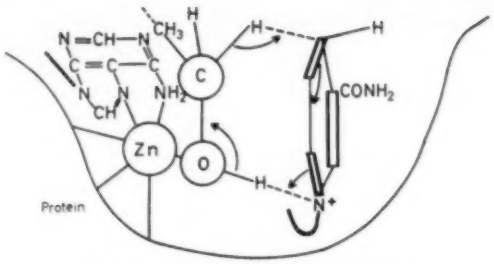


Fig. 3. LADH ternary complexes (schematic)

accept alcohol, caprate or imidazole. DPN changing to DPNH implies a bond flick over, so that *B* (or *C*) becomes free, which only accepts aldehyde, *iso*butyramide or imidazole. Consequently, as a result of a presumably small intermolecular rearrangement, the coenzyme determines which of two bonds is available, each bond determining what can become attached. This concept gains some support from the different structures of the substrates *S* and *S'* and the stereospecific requirement that they change their position somewhat, relative to the enzyme surface[23]. (*b*) *One bond:* bond switching is unnecessary if the coenzymes stay on the same two bonds, but affect the third differently, so that it is a particular type of bond for each. This is indeed the case with the binary complexes and presumably the positively charged pyridinium ring (DPN) allows or directs alcohol, caprate and imidazole on to the zinc bond, while the neutral pyridine ring (DPNH) only allows on aldehyde, *iso*butyramide and imidazole. This mechanism, depending on the different charges of the pyridine (DPNH) or pyridinium (DPN) rings, with one bond and no molecular shifts, is of course attractive, but meets with some difficulties. Perhaps the alcohol hydroxy group takes the place of the water molecule or OH^- involved in the Zn—OH^-—N^+ interaction (Fig. 3). As has been remarked above, the bonds involved in the stability of the complexes are, apart from the zinc–protein bonds, weak to varying extents[24], but this seems necessary as strong bonds result in inhibition rather than catalysis.

Thus in our opinion octahedral zinc is the centre of the ternary complex, although there have been suggestions previously that, like the protein, it is not joined to the substrates[25,26], but to the coenzyme pyrophosphate groups[26]. The tertiary structure of LADH seems to depend on SH-groups[1], and, while the coenzymes bind to the enzyme through adenine, SH-bonds in addition seem necessary with DPNH, but not DPN, where there is the zinc–N^+ interaction. The amide group is not considered joined to the zinc, nor necessarily to the protein at another site, as the protein SH-addition to the pyridine ring in DPNH and the pyridinium–zinc bond in DPN can give the ring its necessary stereospecific rigidity[27].

This mode of bonding gives an explanation of how enzymes cause coenzymes and substrates to select, with great preference, their active reaction partners, while substances like imidazole, which can stimulate reaction at high substrate concentrations and inhibit at low substrate concentrations, could be physiologically significant in having a homeostatic function to maintain substrates at a suitable concentration. An excess would be removed rapidly, but the process would be inhibited strongly when lower concentrations were reached.

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ANTIGEN-ANTIBODY PRECIPITATION CONTRASTED IN GELS OF HIGH AND PHYSIOLOGICAL SALT CONCENTRATIONS

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RABBITS are the usual source of supply of precipitins for serological analyses. The chicken, however, is becoming increasingly popular for this purpose as an animal both readily housed in the laboratory and able to provide volumes of antiserum matching those produced by rabbits[1]. The use of chicken antiserum in precipitin tests performed in liquid medium has led to the discovery that it differs from rabbit antiserum in usually, although not always, precipitating antigen optimally in electrolyte solutions of 10–12 times higher ionic strength than that of physiological saline[2]. At these high electrolyte

concentrations, specific precipitation of antigens by mammalian antisera is grossly inhibited[3]. The recent rise in popularity of chicken antiserum also has extended to its utilization in immunodiffusion tests, that is, in antigen-antibody precipitin tests carried out in gels. We wish to show here that it is false to deduce that if high salt concentrations are optimal for chicken precipitin reactions in liquid media they also must be in semi-solid media. Indeed, not only do physiological electrolyte concentrations seem to offer considerably better conditions than high concentrations for immunodiffusion tests with chicken antisera, but also at high salt concentration in these tests the chicken precipitin reaction is as much inhibited as that of mammalian antisera.

Bovine serum albumin (Armour 'Fraction V' from bovine plasma) was used as antigen for the experiments reported here. Although in this, albumin is the predominating antigen, immunodiffusion experiments with strongly hyper-immune antiserum show that it contains at least 6 other independent antigens (Fig. 1). Since the reaction of each of these with its particular antibody can be resolved and identified in these tests, our results apply at least to these 6 independent antigen-antibody systems examined simultaneously under identical conditions. By chance it happens that at least 2 other antigens also are involved, as constituents of chicken serum (Fig. 1, arrow), bringing the total of antigen-antibody systems at least to 8.

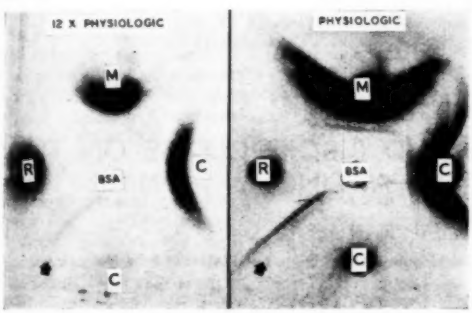

Fig. 1. Comparison in micro-immunodiffusion tests of specific precipitation produced against bovine serum albumin (*BSA*) by chicken (*C*), mouse (*M*) and rat (*R*) antisera at twelve times physiological and at physiological electrolyte concentrations. Arrow points to precipitin bands formed between rat serum and a chicken serum and presumably due to antigens found in the latter, for the rat had been immunized, in addition to *BSA*, with chicken ovalbumin, a preparation known to contain antigens in common with adult chicken serum

Mammalian antiserum is represented by mouse and rat sera ; in respect to the purposes of the present investigation we have found them equivalent to comparable antisera obtained from rabbits and guinea pigs. Serum from these two species was used partly to emphasize the not widely known fact that both are useful precipitin producers. Animals from which antiserum was obtained had been vaccinated subcutaneously with water-in-oil emulsions of bovine serum albumin containing living avirulent (*H37Ra* strain) tubercle bacilli as adjuvant. For example, an animal was injected with 0·1 ml. of emulsion containing 1 mgm. of antigen and 1 mgm. of bacilli. The injection was repeated a week later, and bleedings for antiserum were performed two weeks after the second injection. Our basic requirement was that an antiserum produce readily detectable specific precipitate with its antigen, and it has been satisfied most regularly by this particular immunizing procedure. However, several variations in vaccination protocol have been made, including deletion of water-in-oil emulsion during vaccination, without altering the basic facts pointed out here regarding mammalian antiserum and salt concentration in precipitin tests.

Chicken antisera were obtained by essentially the same procedures, although antigens were used in quantities five times larger. Again, however, vaccination procedures purposely were varied, including whether emulsions were used, to learn whether they conditioned qualitatively the results which were obtained. As among mammalian antisera, they did not. For experiments reported here, two chicken antisera were used, one of high precipitin content and the other with a content barely detectable in micro-immunodiffusion tests.

The crux of our experiments was to discover whether chicken antiserum which precipitates antigen better in aqueous medium at high electrolyte concentrations than at low also would in semi-solid medium, and how this would compare with findings for typical mammalian antisera. Therefore, it was necessary to show that chicken antisera which were to be used did indeed form more precipitate with antigen in high electrolyte concentrations and that mouse antiserum precipitated antigen best at physiological concentration. Optimal proportions for test antisera with antigen were determined at physiological and twelve times physiological salt concentration by timing the appearance of precipitates at the interfaces, in the classic ring test in aqueous medium, in series of tubes in which constant quantities of antisera were overlaid with varying dilutions of antigen. In both this and most immunodiffusion tests, the *p*H of diluent was held at 7·4 with sodium barbital buffering[4]; electrolyte strength was varied solely by changes in sodium chloride concentration. Then, 1 ml. volumes of antigen and antibody, used at their predetermined optimal precipitating ratios, were mixed in centrifuge tubes and allowed to stand for 1 hr. at room temperature and then for 12 hr. at 4° C. Specific precipitate was centrifuged out at 4° C. and 41,000*g* applied for 30 min., washed twice with the same kind of buffer as used for specific precipitation, and quantitated with Boyd's technique for the Folin–Ciocalteu phenol reagent[3]. These tests established that mouse antiserum yielded 20 per cent as much specific precipitate at high salt concentration as at physiological, while chicken antiserum yielded 5 times as much at high as at low. With the classic responses of these respective antisera clearly established, their respective activities in immunodiffusion tests were next investigated.

Numerous immunodiffusion tests, most of them of micro type[5], were set up using these and other antisera and varying for them not only electrolyte concentrations but also the time of reaction development, temperature (4° or 24° C.), type of buffering agent, purity of agar, and type of gel. The results from these experiments were consistent: like mammalian antiserum, chicken antiserum produces heavier precipitin bands in larger numbers and more rapidly at salt concentrations equal to or close to physiological than it does at concentrations 10 or 12 times this, in which by contrast they react so well in aqueous media. Typical findings are illustrated in Fig. 1.

Our findings agree with those recently shown photographically for chicken antisera by Makinodan

et al.[6], who also used bovine serum albumin antigen, but they are at variance with the assertions of Goodman[7] regarding the detection of certain human γ-globulin antigens with such antisera. Although possibly chicken antisera precipitate certain antigens in semi-solid media better at high salt concentrations than at low, just as they obviously do in liquid media, the present experiments suggest that this is the exception rather than the rule. Thus, none of the several antigen-antibody systems shown in Fig. 1 precipitated better (usually not at all) at high salt concentration. Precipitin band curvature for these suggests[1] that the molecular weights of antigens involved ranged from that of bovine serum albumin through that equivalent to mouse, chicken, and rat antibodies (that is, 65,000–180,000). However, in more extensive experiments at present being carried out, we are examining the possibility that human or other γ-globulin antigens reacting with chicken antiserum may indeed, because of some peculiarity of their own, precipitate better in immunodiffusion tests, as in aqueous precipitin tests, at high rather than at low electrolyte concentrations.

At present, we attribute the difference which we have found between liquid and gelled media in connexion with salt concentrations and chicken antiserum precipitin effectiveness to at least two significant differences between the physics of specific precipitation in the two media. One is that in the former, antigen and whole antiserum mix all at once mechanically, by convection, and by diffusion, while in the latter antigen and antibody, separated to some extent from other serum constituents, mix only by diffusion. The other is that in a gel antigen-antibody complexes probably are immobilized shortly after they form, so that if they have tendencies either to dissociate again or to cease growing larger, whence they might fail to precipitate in aqueous medium, these are counterbalanced by surrounding gel[8].

A few additional remarks concerning our experiments are appropriate. Inhibition of specific precipitation by mammalian and chicken antisera at high salt concentrations in semi-solid media occurs in both macro and micro double diffusion tests, although it is more obvious in the latter test because of its ability to resolve more individual precipitin bands. This inhibition occurs to similar extents in highly purified[4] as well as unpurified Difco 'Bacto' agar and in gelatin, and it is present in either barbital- or phosphate-buffered saline solutions. Superficially it seems to be greater in barbital-buffered solutions because these tend to provide resolution of precipitin bands superior to that realized with phosphate-buffered solutions.

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ACTION OF DEOXYRIBONUCLEASE AND RIBONUCLEASE ON THE GROWTH OF EHRLICH ASCITES CARCINOMA IN MICE

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THERE are few reports in the literature on the action of enzymes on cell growth. In tissue cultures, addition of deoxyribonuclease (DNAase) and ribonuclease (RNAase) affected the mitotic rate and the metabolism of the nucleic acids[1–6]. Haddow *et al.* have shown that *in vivo* xanthine oxidase had a definite action on the growth of spontaneous mammary tumours in mice[7,8] and Ledoux and Bergel have demonstrated that tumour growth was depressed by ribonuclease[9–11]. In relation with the above-mentioned facts, it is of interest that DNAase and RNAase activities could not be demonstrated in a variety of experimental and human tumours[12]. An *in vivo* study of the effect of DNAase, RNAase and xanthine oxidase on regenerating rat liver has shown that DNAase and xanthine oxidase affected significantly the proportions of the various mitotic phases whereas ribonuclease did not[13]. This situation prompted me to investigate the effect of DNAase on growth of tumours and to re-investigate further that of RNAase.

Swiss mice of an average body-weight of 20 gm. were used. Ehrlich carcinoma in ascites form was transplanted intraperitoneally using 0·5 ml. of ascitic fluid containing an average number of 65 million cells. The total number of cells was determined as follows : the mice were killed by breaking their necks, their abdomens were skinned and a needle was introduced at the top of the abdominal cavity. The ascitic fluid was drawn out with a syringe, and the cavity was washed four times, once with 2-ml. and then with 1-ml. portions of 0·85 per cent sodium chloride solution. The ascitic fluid plus the washing liquid was adjusted to an exact volume and the cells were counted in an hæmatocymeter after staining with orcein[14].

Smears of ascitic fluid were prepared for metaphase counts and cytological examinations. They were stained by the Feulgen method[15] and, on some occasions, counterstained with methyl green[16]. At least 3,000 cells were counted at random to determine the incidence of metaphase.

In all the assays reported here the control mice were injected intraperitoneally daily with 0·5 ml. 0·85 per cent sodium chloride in a similar manner. Each morning the deaths were recorded in order to establish the survival-curves of both the control and the treated mice.

Deoxyribonuclease. Fig. 1 shows the percentage number of surviving mice in relation with time. The control and treated mice were injected daily from

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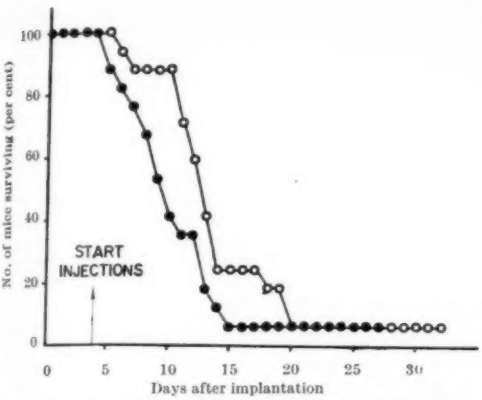

Fig. 1. Effect of injections of DNAase on Ehrlich ascites carcinoma. Mean life: controls, 9·8 ± 3·2; treated, 13·1 ± 3·5. Increase, 34 per cent

the fourth day after implantation of the tumour except on the tenth and seventeenth day. There were 17 mice in each group. The curves show that when 50 per cent of the controls were dead, 88 per cent of the treated mice were still alive. The mean life of the controls was 9·8 ± 3·2 days whereas the treated mice lived on the average 13·1 ± 3·5 days. This increase of 34 per cent in the survival-time was statistically significant at a level better than 98 per cent.

In each group there was a mouse still living 33 days after the transplantation of the tumour. These mice were killed and examined. The mouse in the control group did not show any apparent sign of tumour whereas the mouse of the treated group had a tumour mass of 1·5 cm. in diameter.

Fig. 2 shows the survival-curve of the control and treated mice for an experiment in which the treatment was started on the day following the transplantation of the tumour. The groups contained 10 and 9 mice respectively and the animals were injected daily. When 50 per cent of the controls were dead, all the treated mice were alive. The mean life of the controls was 7·2 ± 2·9 days whereas the treated mice lived 12·0 ± 3·6 days. This increase of 67 per cent in the survival-time is statistically significant at a level better than 99 per cent.

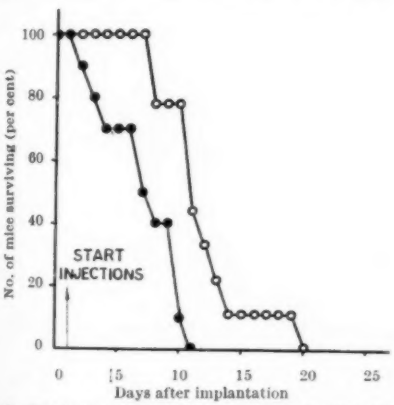

Fig. 2. Effect of injections of DNAase on Ehrlich ascites carcinoma. Mean life: controls, 7·2 ± 2·9; treated, 12·0 ± 3·4. Increase, 67 per cent

Thus the injection of DNAase one day after the transplantation of the tumour instead of the fourth day doubles the increase in survival-time.

From these results it would seem that DNAase exerted an action on the growth of Ehrlich ascitic carcinoma, and that the magnitude of this action depended on the time the injections were started following the transplantation of the tumours.

The action of DNAase on the growth of Ehrlich ascites tumour cells was also ascertained by counting the total number of cells in control and treated mice. The incidence of metaphase was also determined. The mice were injected daily for four days starting the day following the transplantation of the tumour. Table 1 shows that the total number of cells in the treated mice was one-third that of the control mice. The number of cells in the controls, from the day of transplantation to the fifth day, increased nearly six times whereas in the treated mice it increased only four-fold. The incidence of metaphase in the treated mice was only one-half that in the controls, indicating that cell division was impaired in the presence of the injected DNAase.

Table 1. Effect of DNAase on Cell Number and Incidence of Metaphase in Ehrlich Ascites Carcinoma

Animals	Days	Total No. of cells ($\times 10^{-4}$)	Incidence of metaphases
Controls	0	56	1·5 ± 0·3
	5	320	
Treated	0	56	
	5	230	0·7 ± 0·3

Daily injections of enzyme started the day following the implantation of the tumour.

The action of the injected DNAase on the deoxyribonucleic acid of the cells was followed by Feulgen staining. Examination of Feulgen-stained smears of tumour cells from control and treated mice indicated that the nuclei of the tumour cells from the treated mice were not stained at all or were much less stained than those from the controls. This shows that some deoxyribonucleic acid of these cells was most probably destroyed by the enzyme. A large number of cells also contained Feulgen-positive material in their cytoplasm.

These facts seem to indicate that the injected DNAase acts on the growth of the tumour, impairing cell division by attacking deoxyribonucleic acid. Similar results were obtained by Chevremont *et al.*[2-5] on the action of DNAase in tissue culture. These authors reported that DNAase impaired the growth of fibroblasts and myoblasts of chicken embryos in tissue culture and stopped cell division. A lack of DNA was observed in the nucleus and some found in the cytoplasm.

The activities of five enzymes of the purine-catabolizing enzyme system have been determined as previously described[17] in control and treated mice to gain information on the possible effect of DNAase injections since the enzyme seemed to depolymerize the deoxyribonucleic acid of the cell. Table 2 shows that the activities per average cell of 5-adenylic acid nucleotidase, inosine phosphorylase, guanase, xanthine oxidase and uricase were very low in Ehrlich ascites tumour cells, even lower than in primary liver tumours or Novikoff hepatoma[17,18]. Injections of DNAase for four consecutive days did not seem to affect the activities of these enzymes except for 5-nucleotidase, which was increased about

Table 2. Effect of DNAase on Purine Catabolizing Enzymes in Ehrlich Ascites Carcinoma

Enzymes	Activity in μM/min./cell ($\times 10^8$) Controls	Treated
Uricase	0	0
Xanthine oxidase	0·021 ± 0·013	0·024 ± 0·009
Guanase	0·24 ± 0·10	0·21 ± 0·11
Inosine phosphorylase	1·43 ± 0·37	1·14 ± 0·42
5-nucleotidase	0·38 ± 0·21	2·13 ± 0·70

six-fold. However, this activity was still so low that the significance of this increase was questionable. It would thus seem that, under the present conditions, the catabolism of purine or purine derivatives was not affected.

Ribonuclease. Fig. 3 shows the percentage number of surviving mice in relation with time. The injections were started the day following the transplantation of the tumour. Both groups contained thirty mice, and the animals were injected daily. It can be seen from the curves that the period of 50 per cent death was the same for the two groups. The mean life of the controls was 8·3 ± 3·9 days whereas the treated mice lived on for 10·1 ± 4·5 days. This increase of 22 per cent in the survival-time was not statistically significant.

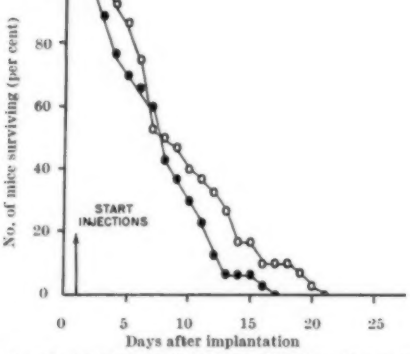

Fig. 3. Effect of injections of RNAase on Ehrlich ascites carcinoma. Mean life : controls, 8·3 ± 3·9 ; treated, 10·1 ± 4·6. Increase, 22 per cent

To ascertain that the difference between the action of RNAase and DNAase was not due to a lack of penetration of the RNAase in the cells, ribonuclease activity of the ascites cells and of the ascitic fluid from control and treated mice has been determined. Immediately after withdrawal of the fluid with a syringe, the cells were centrifuged and re-suspended in sodium chloride, and RNAase activity was determined on both cell suspension and supernatant as previously described[19]. Table 3 shows that the RNAase activity, either at acid or alkaline pH, was the same for both the supernatant and the cell re-suspension for the controls. For the treated mice, however, the enzyme activity of the cell re-suspension was more than twice that of the supernatant. Furthermore the ribonuclease activity, measured at acid pH, of the treated cells was nearly five times that of the control cells, and the activity, measured at alkaline pH, nearly seven times. This indicated that the injected enzyme penetrated inside the cells as shown by Ledoux and Pileri[20] and that the lack of effect on tumour growth was not due to a lack of penetration of the enzyme.

Table 3. Effect of RNAase Injection on the Enzyme-Level of the Cells

	Ribonuclease activity* Acid		Alkaline	
	Controls	Treated	Controls	Treated
Supernatant	0·070	0·127	0·110	0·426
Cells	0·058	0·262	0·134	0·902

* Expressed in O.D.

The lack of action of RNAase on the growth of Ehrlich ascites carcinoma does not confirm previous results of Ledoux, who reported significant effect of the enzyme on the growth of this tumour[9]. However, this might be due to a different source of RNAase since Ledoux has shown that the biological activity of different RNAases from various sources varied tremendously. Furthermore, the tumour inoculum, in the present work, was much larger than that employed by Ledoux and the injected dose of RNAase was much smaller.

Conclusions. Injections of DNAase increased the survival-time of mice bearing the Ehrlich ascites carcinoma by 34 and 67 per cent, depending on the time the injections were started following transplantation. This increased survival-time was accompanied by a decreased metaphase index and by the disappearance of deoxyribonucleic acid from a number of cell nuclei. The injections of DNAase had no effect on the activities of five enzymes involved in the catabolism of purine and purine derivatives.

Injections of RNAase had no significant action on the survival-time of mice bearing Ehrlich ascites tumour. This lack of action was not due to a lack of penetration of the enzyme inside the tumour cells.

The advice of Dr. R. Daoust for the cytological examinations is gratefully acknowledged. The work has been supported by grants to Dr. A. Cantero, director of the Research Laboratories, from the National Cancer Institute of Canada.

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VACUOLATION AND LEAF REGENERATION IN *SPLACHNUM AMPULLACEUM* (L.) HEDW.

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Formation of Secondary Vacuoles in De-differentiating Cells

It was shown in ref. 1 that cellular de-differentiation in *Splachnum* involves profound changes in the intracellular configuration; one of the first signs of this is the formation of intravacuolar cytoplasmic lamellæ leading to systrophe. This communication is concerned with the formation of these intravacuolar cytoplasmic layers and some considerations arising from these observations.

Plant material and methods are the same as described in ref. 2. Leaves isolated from three-week-old plants were, however, transferred into liquid Beijerinck solution adjusted to *p*H 6·8. Regeneration takes place readily from isolated leaves at this *p*H.

Fig. 1 represents a composite drawing of a leaf cell in cross-sectional view parallel to the leaf surface showing various stages in the development of the primary cytoplasmic lamellæ. It is evident that these lamellæ originate in the form of secondary vacuoles. These vacuoles are formed in various parts of the peripheral cytoplasm, predominantly in the vicinity of the nucleus. One secondary vacuole expands initially into the central vacuole and moves about considerably until it reaches ultimately the peripheral cytoplasm across the vacuole. Fusion of separate intravacuolar cytoplasmic layers appears to take place not infrequently. Lamellæ may originate also in the form of a secondary vacuole from the peripheral cytoplasm opposite the nucleus expanding toward the nucleus. Secondary vacuolar formation continues over considerable periods of time from other sites of the peripheral cytoplasm during and after the formation of the primary intravacuolar cytoplasmic lamellæ.

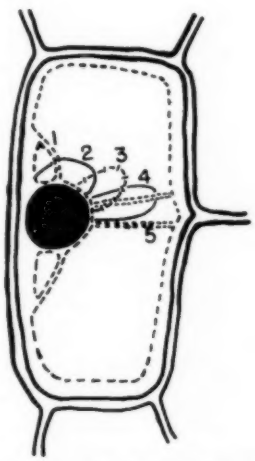

Fig. 1. Composite drawing of leaf cell (marginal position) showing formation of primary intravacuolar cytoplasmic lamellæ. The numbers represent successive stages in the expansion of a secondary vacuole

The pattern described is characteristic of cells near the leaf margin. In other portions of the leaf, only one primary intravacuolar lamella is formed initially, originating in the form of a secondary vacuole from the peripheral cytoplasm, frequently not in the vicinity of the nucleus.

The similarity between secondary vacuole formation in de-differentiating cells and that in plasmolysed cells should be noted. Haberlandt[3–5] observed intravacuolar lamellar structures in plasmolysed cells and his observations were fully confirmed by those of Pfeiffer[6]. Both Haberlandt and Pfeiffer found cellulose incorporation into the intravacuolar lamellæ. Küster[7] has been unable to confirm this incorporation, and doubts, therefore, that intravacuolar formation of lamella has anything to do with cross-wall formation or cell-division. Although the present work has not been concerned with this question, cellulose incorporation is unlikely to occur up to the time of phragmosome formation and would appear to be restricted to it. Haberlandt[5] suggested, on the basis of his interpretation of the nature and fate of the lamellæ, that divisional substances are normally present in cells which increase in concentration following the decrease in water concentration resulting from the plasmolytic treatment of the cell. Division, according to Haberlandt, may set in, once a critical concentration in divisional substances has been reached. The present results make this explanation unlikely since plasmolysis and, therefore, loss of water do not appear to take place and since primary intravacuolar cytoplasmic lamella formation does not represent division. Pfeiffer[6] concluded on different grounds that Haberlandt's proposed mechanism can at least not be the primary one.

Küster[8] has discussed the ontogeny of intravacuolar lamellæ in plasmolysed cells and concluded that they are the result of a process of furrowing of the primary vacuole and do not arise as a result of secondary vacuole formation from the peripheral cytoplasm. This conclusion cannot be maintained considering the present results and those of Wartenberg[9]. Wartenberg has recently given strong evidence that plasmolysis represents an active swelling process, that is a process of active vacuole formation in the outer peripheral cytoplasm. The present observations on early stages of secondary vacuole formation resemble Wartenberg's on secondary vacuoles arising in plasmolysed cells. Similarities between changes in the intra-cellular configuration of de-differentiating cells and that of plasmolysed cells have been noted earlier[2]. The resemblance between the formation of a secondary vacuole in plasmolysed cells and in non-plasmolysed but de-differentiating cells stressed in the present discussion appears to be significant. It may be suggested that plasmolytic treatment results in the initiation of cellular de-differentiation. This is supported by observations, for example, Isaburo, Nagai[10], that plasmolysed cells may regenerate, that is de-differentiate and re-differentiate, when the plasmolytic treatment is followed by deplasmolysis. Secondary vacuole formation would then

generally represent early stages in the process of cellular de-differentiation. It may not be sufficient to explain the colloid chemical behaviour of the cell in terms of an interaction between the cell and the environment (for example, plasmolyticum); it may rather have to be considered also in terms of the interaction between different cells or the lack of it, that is, in terms of the correlative influences between different parts of the organism.

Effect of Kinetin on Attached Leaf Regeneration

It has been shown recently[11] that the realization of regenerative potentialities in cells of a leaf attached to a stem segment isolated from a gametophore is inhibited by this stem segment, while the presence of the attached leaf increases regeneration from cells of the stem segment. In the course of an investigation into the nature of this correlation system, the effects of locally applied kinetin have been investigated.

Stem segments with one attached leaf from which the distal third was removed were isolated from the middle region of the gametophore, one segment for each gametophore. They were planted on Beijerinck's medium solidified with 2 per cent agar with various concentrations of kinetin added, only the region near the wound surface of the attached two-thirds leaf being in contact with the medium. They were grown under continuous white light provided by daylight fluorescent lamps. Gametophores used were always 20–21 days old.

The number of protonematal regenerates for the attached two-thirds leaf was determined three days following planting and the results are shown in Fig. 1. Regeneration is almost absent in attached leaves planted on the medium without kinetin. In the presence of certain kinetin concentrations, however, attached leaf regeneration is promoted. It should be noted that the effective concentration range shown in Fig. 2 was obtained only with freshly dissolved kinetin. Kinetin dissolved and kept in the refrigerator showed effectiveness only at higher apparent concentrations. Kinetin locally applied to the distal end of the attached leaf may therefore overcome, to some extent, inhibition of attached leaf regeneration. The stem segment represents apparently a region of attraction for substances essential for regeneration and produced predominantly by the leaf. The drainage of the leaf by the stem prevents attached leaf regeneration while it promotes regeneration from the stem segment. Basipetal transport of these substances is locally counteracted in the leaf by kinetin application, transport taking place also toward the site of kinetin application leading to regeneration in cells near this site.

This suggestion is supported by the following experimental observations. When intact gametophores are grown on Beijerinck's medium containing twice the normal ammonium nitrate concentration, increased regeneration takes place from a two-thirds leaf attached to a stem segment in the absence of kinetin. It appears that in this case nitrogenous compounds are produced at higher concentrations and that basipetal transport of these substances from the leaf toward the stem segment does not lead to concentrations in the leaf limiting regeneration.

The present concept of the nature of the correlation system and kinetin action described here is supported by the original observations by Richmond and Lang[12]

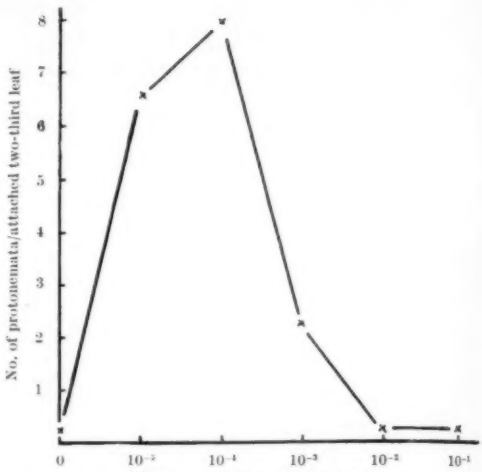

Fig. 2. Protonematal regeneration from two-thirds leaves attached to a stem segment in the presence of various concentrations of kinetin

and the extensive investigations by Mothes *et al.*[13] on the effects of kinetin on the directional transport of soluble nitrogenous compounds. Localized application of kinetin in isolated leaves of *Nicotiana rustica* led to a localized accumulation and retention of soluble nitrogen compounds against concentration gradients, these substances being available for renewed protein synthesis. Since regeneration involves increased synthetic activities, particularly in terms of proteins (see ref. 14 for present material), such accumulation would appear to be significant also for regeneration. In Heitz's view[15], accumulation of sugars is the most important factor for the initiation of regeneration in isolated leaves. Although regeneration did not result, he obtained increases in nuclear size by applying sucrose to leaves. Stange[16] observed a dependence of increases in size of nucleus in regenerating cells of *Riella* on length of periods of photosynthesis.

The present interpretation of the system described here may have wider applications for correlations inhibiting the realization of regenerative potentialities and the distribution of processes of de-differentiation taking place in normal plant development[14].

This work was supported by a grant from the National Research Council of Canada.

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LETTERS TO THE EDITORS

COSMOLOGY

Dirac's Cosmology

DIRAC's cosmology was based on the principle "that any two of the very large dimensionless numbers (of the order 10^{39} and 10^{78}), occurring in Nature, are connected by a simple mathematical relation in which the coefficients are of the order of magnitude unity"[1]. From this principle Dirac deduced that the gravitational 'constant' $G \propto (\text{epoch})^{-1}$. In order to describe a cosmological model according to the principles of the general theory of relativity he then changed the units of distance and time so as to make G constant. With the new units the metric can be written:

$$ds^2 = c^2dt^2 - R^2(t)\,(dx^2 + dy^2 + dz^2) \qquad (1)$$

with $R(t) = t^{2/3}$. Afterwards I considered this model, and by making certain assumptions, which appeared reasonable, obtained $G \propto \frac{1}{t}$, $t \approx 4 \times 10^9$ years[2]. This gives the values:

$$H \approx 160 \text{ km./sec./}10^6 \text{ parsec.}, \quad q = -0{\cdot}5 \qquad (2)$$

for Hubble's constant $H\left(=\frac{\dot{R}}{R}\right)$ and the acceleration parameter $q\left(=\frac{R\ddot{R}}{\dot{R}^2}\right)$.

Recently, L. Egyed[3] has proposed a theory of the origin of the solar system which uses these results $\left(G \propto \frac{1}{t},\ t \approx 4 \times 10^9 \text{ years}\right)$, and P. Hédervári[4] has used the same results in a theory of the internal structure of the Moon. My main object in this communication is to point out that the assumptions made in these interesting theories are not necessarily associated with the values of H and q given above, but may also give the values:

$$H \approx 80 \text{ km./sec./}10^6 \text{ parsec.}, \quad q = -2 \qquad (3)$$

from a different description of the Dirac model in Riemannian space-time. It seems advisable to point this out, since the empirical values of H and q may soon be sufficiently accurate to be able to distinguish between (2) and (3).

Rindler[5] shows how cosmological models, including those which are not described according to the principles of the general theory of relativity, may be described in Riemannian space-time, and he gives a metric for the Dirac model of the form (1) with $R(t) = t^{1/3}$. This method of discussing the model is based on the results derived from Dirac's principle, and does not provide a theory of gravitation for the model. Recently, however, I have shown that a theory of gravitation with $G \propto \frac{1}{t}$ may be based on field equations derived from a principle of stationary action[6]. In that theory the characteristic metric was (1) with $R(t) = t^{1/3}$, and the gravitational theory may therefore be associated with the Dirac model. If one assumes $t \approx 4 \times 10^9$ years, this description of the Dirac model gives (3) for H and q.

When one considers the empirical values of H and q it is of interest to observe that McVittie[7] discussed the observational results available up to July 1955 and found values for H of 143–227 km./sec./10^6 parsec. Later, Sandage[8], in studying galaxies in the Virgo cluster, found that Hubble mistook glowing H II regions for brightest stars and, by applying corrections to Hubble's data, found that H can range between 50 and 100 km./sec./10^6 parsec. with a very large uncertainty. De Vaucouleurs[9] suggested that the local group is part of a super cluster of galaxies, and, by making allowance for the velocity of our own system which would occur on account of the differential rotation and expansion of the cluster, he obtained the value $H \approx 140$ km./sec./10^6 parsec. The empirical value of q is also very uncertain at present. Humason, Mayall and Sandage[10] give $q = -3{\cdot}0 \pm 0{\cdot}8$ and Baum[11] finds from photoelectric determinations of red shifts that q has a value in the range $-0{\cdot}5$ to $-1{\cdot}5$.

Thus it would appear that no definite conclusions can be reached from consideration of the empirical results, and that the values of H and q given by either (2) or (3) are within the ranges of these results.

Finally, consider the assumption that $t \approx 4 \times 10^9$ years, in relation to some recent astrophysical calculations. Hoyle[12] has estimated the time required for the evolution of certain stars as $> 10^{10}$ years, but in his calculations he has taken G = constant, whereas in the Dirac model $G \propto \frac{1}{t}$. Hoyle's figures are not, therefore, strictly comparable with the assumption that the age of the universe $\approx 4 \times 10^9$ years. This age is, however, too small to allow time for the synthesis of the heavy isotopes of uranium in a star ($\approx 6{\cdot}6 \times 10^9$ years), by the process described by Burbidge, Burbidge, Fowler and Hoyle[13].

I am indebted to Dr. G. J. Whitrow for information concerning the empirical results.

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RADIOPHYSICS

Spectral Characteristics of Type II Solar Radio Bursts

It has been recognized for some time that Type II solar radio bursts are due to the excitation of plasma oscillations in the solar corona by some exciting agency which moves through the corona. Roberts[1], in reviewing observations and theories of these phenomena, leaves unexplained two salient characteristics: (1) the appearance of radiation at the fundamental and second harmonic at comparable powers and the lack of radiation at all higher harmonics; (2) the frequent splitting of each of these two 'lines' into a pair of lines, the fractional splitting of each of these two pairs being the same, typically a few per cent.

Plasma oscillations are inherently non-radiative, so that we must ascribe our observation of these oscillations to their coupling to electromagnetic waves. In a homogeneous plasma, such coupling occurs only through non-linearity of the field and dynamical equations. It has been pointed out elsewhere[2] that the dominant coupling process is between two plasma-oscillation modes and one electromagnetic wave, which leads to radiation at $2\omega_p$, that is, twice the plasma frequency ω_p, where $\omega_p^2 = 4\pi ne^2/m$, and that this is the key to understanding of point (1). The next most important non-linear process couples three plasma-oscillation modes and one electromagnetic wave, and leads to radiation at ω_p and at $3\omega_p$ with comparable intensities. Since the third harmonic is not observed, we may conclude that the plasma-oscillation amplitude (measured as a fractional change in electron density) is small, and that emission at the fundamental is not due to non-linearity. Radiation at the fundamental may be ascribed to inhomogeneity of the coronal plasma, due to thermal fluctuations, to turbulence, or to a shock wave associated with the exciting agency.

In order to understand point (2), we should now consider the plasma to be permeated by a magnetic field. In the 'electrostatic' approximation, $\omega/k \ll c$, the dispersion relation has the form:

$$\omega^2 = \tfrac{1}{2}\left[\omega_p^2 + \omega_g^2 \pm (\omega_p^4 + \omega_g^4 - 2\omega_p^2\omega_g^2\cos 2\theta)^{1/2}\right]$$

where θ is the angle between the wave vector k and the magnetic field vector B, and ω_g is the electron gyro-frequency eB/mc. It is noteworthy that ω does not depend on $|k|$. For $\omega_g < \omega_p$, this separates into two bands, one extending from 0 to ω_g and the other from ω_p to $(\omega_p^2 + \omega_g^2)^{1/2}$, the former of which one would not expect to radiate through the corona. Since one observes two closely spaced frequencies rather than a wide band, we must inquire whether some modes of the upper band are more strongly excited than others.

The significance of group velocity in the problem of the excitation of waves in a plasma by a moving source of finite dimensions has been recently discussed[3]. The problem may be regarded as that of the excitation of a resonator by an aperiodic force, and we know that the excitation varies proportionately with the Q of the resonator. Modes of highest Q will be those which lose their energy most slowly. If the wave-number spectrum and the dimensions of the source are subject to certain limitations, then loss of energy radiation, by electron-ion collisions and by Landau damping, will be subordinate to loss of energy by convection, a process which is characterized by the group velocity. We then expect that the most highly excited modes will be those of lowest group velocity.

By setting $\partial\omega/\partial k_x = \partial\omega/\partial k_y = \partial\omega/\partial k_z = 0$, we may find the modes of zero group velocity. Since ω here depends only on θ, the condition reduces to $d\omega/d\theta = 0$, which is satisfied at $\theta = 0$ and at $\theta = \pi/2$. Hence, considering only the upper band, we should expect the frequencies ω_p and $(\omega_p^2 + \omega_g^2)^{1/2}$ to be preferentially excited. It is proposed that these are the two frequencies to be identified with the pair of frequencies of the observed spectrum when it is split. The separation is seen to be approximately $\frac{1}{2}\omega_g^2/\omega_p$. This leads typically to estimates of 20 gauss for the strength of the magnetic field in the region associated with the beginning of a Type II burst.

The above interpretation of the spectral characteristics of Type II bursts would make it possible to obtain, from observations, information about the magnetic field in the solar corona.

The observed frequency ratio of 'first harmonic' to 'fundamental' is slightly less than 2:1. This effect has been attributed[4] to the propagation characteristics of the corona. It may be due in part to differences between the coupling mechanisms: for certain forms of the plasma oscillation and inhomogeneity spectra, one finds that radiation at the fundamental is due to a band of wave numbers, and hence of frequencies, higher than that due to radiation at the second harmonic, thermal velocities of the plasma electrons now being taken into account.

An amplified version of the above arguments and a discussion of the nature of the exciting agencies of Type II and Type III bursts will be given in a forthcoming article.

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Decimetre Radio Bursts Concurrent with Solar Type IV Radiation

It is known that when there is an important H_α flare on the Sun's disk there is often considerable radio emission over a wide band of frequencies. While the spectrum at metre wave-lengths is relatively well understood, the decimetre and centimetre radio bursts have not been related very clearly to the overall spectrum.

Especial interest is attached to the broad-banded centimetre and decimetre radiation that occurs concurrently with the metre wave-length continuum emission, known as Type IV radiation, and thought to be 'synchrotron' emission. Takakura[1] suggested that the microwave emission might be an extension of the metre wave-length Type IV spectrum to shorter wave-lengths.

From many observations of 1,420-Mc./s. solar radio bursts with the Christiansen crossed-grating interferometer used to provide a fan-beam (2′ of arc to half-power in the east–west direction) we have chosen for study eight occasions characterized by intense Type IV activity at metre wave-lengths.

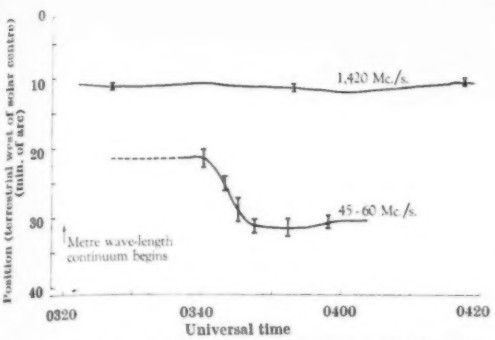

Fig. 1. Position of decimetre and metre wave-length sources, July 29, 1958

Accurate estimates of position were obtained at 4-min. time intervals, and were compared with the position of the Type IV source in the frequency range 45–60 Mc./s. While the metre wave-length sources were located in the high corona, the 1,420-Mc./s. sources remained at rest in the chromosphere or lower corona, probably at the same height as the H_α flares and certainly not more than about 50,000 km. above them. The positions of the microwave burst and the flare were always closely coincident. The low-frequency sources showed large-scale movements (presumably outward) on two occasions (one of which is shown, Fig. 1), while the decimetre source was stationary.

Typical sizes of the 1,420-Mc./s. sources were 2′–5′ of arc, and brightness temperatures of 10^8–10^9 ° K., were observed concurrently with the Type IV continuum at lower frequencies, indicating a non-thermal mechanism at decimetre wave-lengths.

Since the decimetre burst and the metre wave-length Type IV source are located at widely different parts of the solar atmosphere they must be different. This conclusion supports a recent view of Takakura and Kai[2], who suggest, from spectral considerations at 4,000-Mc./s., that the microwave bursts "are not the high-frequency component of Type IV bursts on metre-waves but another distinctive type of burst emitted from radio sources fixed in the coronal condensations".

These results were first presented at an Institute of Physics Symposium (Brisbane, May, 1961).

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PHYSICS

Gravitational and Electrostatic Forces

In a recent communication[1], it was pointed out that dimensional difficulties met with in the usual gravitational equation $F = GM^2L^{-2}$, where F is the attractive force and G the gravitational constant, are avoided if the mass of a body is replaced by N_1e, where N_1 is the number of positive (or negative) charges contained in the body and e is the elementary charge. It then follows that for two bodies placed a distance r apart and containing N_1 and N_2 charges, respectively, $F \propto N_1N_2e^2r^{-2}$.

For two electrons, the ratio between their electrostatic repulsion and their gravitational attraction is close to $4{\cdot}2 \times 10^{42}$. Attempts have been made, notably by Eddington, to explain this enormous value, but without much success. It is now possible to make a fresh approach along the lines suggested here.

Consider two very small bodies r cm. apart and each containing 1 gm. of matter, and suppose that each body contains N protons (or electrons), a neutron for our present purpose being regarded as equivalent to one proton and one electron. Then the electrostatic force in dynes between the protons (or electrons) in the two bodies is N^2e^2/r^2 and the gravitational force between the protons is very close numerically to G/r^2. If the ratio of proton mass to electron mass is α, the gravitational force between the groups of electrons in the two bodies is G/α^2r^2, and the ratio of electrostatic force to gravitational force is $N^2e^2\alpha^2/G = R$, where R is the numerical value of this ratio. Thus R/N^2 is a pure numeric, equal to $4{\cdot}23 \times 10^{42}$ divided by $(6{\cdot}023 \times 10^{23})^2$. The result, $1{\cdot}166 \times 10^{-5}$, is equal to $e^2\alpha^2/G$ and it is reasonable to inquire if this value can be derived in terms of the fundamental constants e, h and c, where h is Planck's constant and c is the velocity of light *in vacuo*. Only one dimensionless combination of these constants is known, namely, $2\pi e^2/hc$, equal very nearly to 1/137, and it is natural to see whether this quantity can be utilized. A possible relationship is $\frac{e^2\alpha^2}{G} = \frac{2}{9}\left(\frac{2\pi e^2}{hc}\right)^2$, since $\frac{e^2\alpha^2}{G}\left(\frac{hc}{2\pi e^2}\right)^2 = 0{\cdot}219$ when the experimentally determined values are substituted. Assuming that $\frac{\alpha^2}{G} = \frac{2}{9}\left(\frac{2\pi e}{hc}\right)^2$, it is tempting to split this equation into separate expressions for α^2 and G. One possibility is:

$$\alpha^2 = \frac{4\pi e(q_0 = 1)}{9hc} \text{ or } \alpha = \frac{2}{3}\sqrt{\left(\frac{\pi e}{hc}\right)}, \text{ numerically, and}$$

G (referred to masses of 1 gm.)

$$= \frac{hc\,(q_0 = 1)}{2\pi e} = \frac{hc}{2\pi e}, \text{ numerically}$$

It is necessary to introduce q_0, with the dimensions of charge, into these equations to make them dimensionally correct, and $q_0 = 1$ simply means that q_0 is the charge which when placed 1 cm. away from a similar charge causes an electrostatic force of 1 dyne to act between them, the unit of mass being 1 gm. If the unit of mass is changed, q_0 and G also change, but α is unaltered. Using the best available data for e, h and c, $hc/2\pi e = 6{\cdot}58 \times 10^{-8}$ and $\frac{2}{3}\sqrt{\left(\frac{\pi e}{hc}\right)} = 1{,}837{\cdot}4$.

These results compare with the experimentally determined values $6{\cdot}67 \times 10^{-8}$ and 1,836·1. The slight disagreement between the calculated and experimentally determined values of G is not surprising, since the assumption that mass is proportional to the number of positive (or negative) charges is not strictly true. For hydrogen there are 1·008 mass units for one pair of positive and negative charges, whereas for most of the Periodic Table the ratio is close to 1 mass unit per pair of charges. In every atom but hydrogen, mass is used in stabilizing the nucleus, and since the electrical value of the gravitational constant, $hc/2\pi e$, takes no account of this loss of mass, the value $6{\cdot}58 \times 10^{-8}$ must refer to hydrogen atoms. Now the gravitational force between two ordinary bodies r cm.

XUM

apart may be expressed as $F = GN_1N_2/N^2r^2$, where N in this case is the number of protons in 1 gm. of average matter. For hydrogen N is replaced by $N/1{\cdot}008$, so that $F = (1{\cdot}008)^2N_1N_2hc/2\pi N^2r^2e$. The normal value of G is therefore equal to $1{\cdot}008^2 \times 6{\cdot}58 \times 10^{-8}$, that is, $6{\cdot}68 \times 10^{-8}$, in close agreement with the experimentally determined value.

For two 1-gm. masses of hydrogen r cm. apart, the gravitational force between the electrons is given by $G/(1 + \alpha)^2r^2$ rather than G/α^2r^2, so that the calculated value of α, 1,837·4, should really be equated to $\alpha + 1$. Thus the ratio of proton mass to electron mass becomes 1,836·4, in excellent agreement with the experimental value.

Summarizing, it is possible, apart from minor corrections, to represent the electrostatic force between two charged bodies and also the gravitational force between protons and between electrons by the following equation:

$$F = \frac{n^2N_1N_2e^2}{8kr^2}\left(\frac{hc}{\pi e^2}\right)^{n-1}$$

where n has, respectively, the values 1, 2 and 3 for the three cases in the order given, and k is a dimensionless constant with numerical values as follows:

n	1	2	3
k	1/8	N^2e	N^2

For electrons and protons contained in each case in 1 gm. of matter and placed 1 cm. apart, the gravitational force in dynes is given numerically by:

$$F_{e,p} = \frac{3}{4}\left(\frac{hc}{\pi e}\right)^{3/2}$$

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Radio-Luminescence in Sodium Chloride and Potassium Chloride

Work on radio-luminescence in rock salt and fluorite and other naturally occurring minerals excited by X-rays and nuclear radiations has been reported by some workers and summarized by Przibram[1]. The present communication deals qualitatively with the production and quenching of luminescence in pure and additively coloured crystals of sodium chloride and potassium chloride, excited by X-rays from a copper target.

A narrow beam of X-rays was allowed to enter a lead box impervious to light through a small circular aperture. The crystal was mounted on a holder with 'Apiezon Q', and was centred in such a way that the X-ray beam fell squarely on to the crystal. 'Apiezon' was found to scatter a minimum of X-rays. An E.M.I. 9502 photomultiplier was mounted at right angles to the direction of the X-ray beam, and was made to look on to the crystal through a lead glass window and a shutter. The recording of continuous readings was made possible by such an arrangement, without any interference from the X-ray beam. The microammeter readings at the output of the photomultiplier gave a measure of the luminescence. The photomultiplier was worked with a cathode to first dynode voltage of 160 V. and subsequent interdynode voltage of 80 V.

The crystals were continuously irradiated with 30-keV. X-rays at 10 m.amp., and at various time intervals the luminescence output for different intensities was noted. In Figs. 1 and 2 the luminescence output is plotted against time of exposure for sodium chloride and potassium chloride, at all the intensities used. In both cases the luminescence output is found to rise steeply in the first few minutes and to fall slowly to the saturation value as more and more luminescent centres are destroyed.

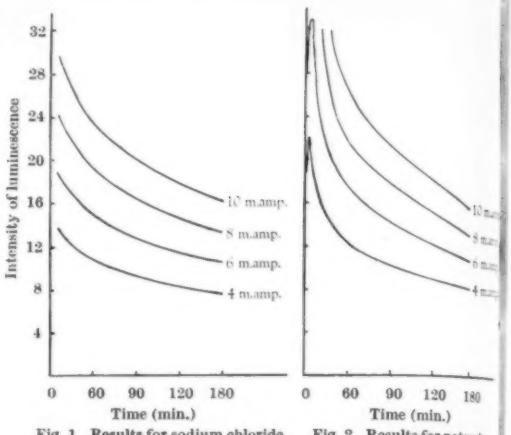

Fig. 1. Results for sodium chloride

Fig. 2. Results for potassium chloride

The luminescence output is plotted against X-ray intensity at different times of exposure, in Fig. 3 for pure sodium chloride and in Fig. 4 for pure potassium chloride crystal and for the same crystal additively coloured with about 10^{17} F-centres/c.c. These plots bring out the interesting feature of the variation of the slopes of these linear relations with the time of exposure. As the time of exposure increases, the slope decreases, and the curves tend to that due to the additively coloured sample, showing that after high times of exposure a crystal irradiated with X-rays reaches a saturation value, where it behaves like an additively coloured crystal; although it can still lose its trapped electrons by exposure to white light. The intercepts of the y-axis gives the level of phosphorescence introduced into the crystal due to irradiation.

Preliminary results with a number of alkali halides, pure and additively coloured, using the photon-counting technique, showed similar behaviour with sodium chloride and potassium chloride. Potassium

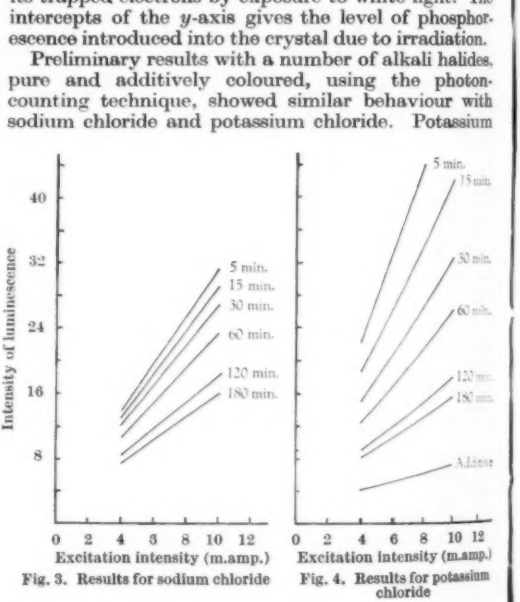

Fig. 3. Results for sodium chloride

Fig. 4. Results for potassium chloride

iodide showed very fast quenching with virtually no phosphorescence, whereas potassium bromide gave excellent response but long-lasting phosphorescence. The deterioration of luminescence efficiency was almost exponential in potassium chloride and rather slow in sodium chloride, showing that quenching centres are produced in potassium chloride faster than in sodium chloride.

Thanks are due to Dr. K. S. Iyengar for his keen interest and constant encouragement during the course of this investigation and to Mr. R. Thyagarajan for the crystals.

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GEOLOGY

Occurrence of Graywacke in the Lower Siwaliks, Simla Hills

We would like to direct the attention of scientists regarding the occurrence of graywacke in the Nahan Series near Kalka, Simla Hills, Panjab, which has remained unnoticed in Indian geology.

During the course of a traverse near Kalka, while studying the terraces of Kaushallia and Jhajjar Rivers, we came across a grey to greenish micaceous 'sandstone' type of rock which in hand specimens appeared to be graywacke. This was confirmed in thin sections of the rocks, which revealed the presence of angular grains of quartz (approximately 50 per cent) embedded in a matrix composed of fragments of slate, schist, quartzite, chert, felspar, chlorite and authigenic sericite. The rock grades imperceptibly into the underlying red clays.

This graywacke is considered to be a part of the lower Siwaliks known as Nahan Series of the area. All the existing text-books on the geology of India state that these deposits are of fresh-water origin. They are considered to be made up of sandstones, grits, conglomerates, clays, and silts having the characters of fluviatile deposits of torrential streams and floods in shallow water basins.

It is interesting to note that as early as 1937, Krynine had referred to the presence of graywacke from the Siwalik formations of north-west India. However, it has failed to direct the attention of the Indian geologists and hence remained out of the present-day Indian geological literature. We would like to state that the present findings were made independently of Krynine's observation.

Although very diverse types of graywacke having variable mineral composition have been described from different parts of the world, it is generally accepted that the graywackes are characteristic of the geotectonic belts. The presence of this rock in the Himalayan foothills is therefore noteworthy as it will throw light on the sedimentation history of the Siwaliks and other adjoining formations at least of this area. It is therefore probable that the notion that the Siwaliks are entirely of fresh-water origin may no longer be true at least in this part of the sub-Himalayan region.

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RADIATION CHEMISTRY

Behaviour of Neutron-irradiated Ammonium Sulphate on Thermal Annealing

The behaviour of a neutron-irradiated substance in thermal annealing is known to depend on the temperature and on the period of heating. Normally, the retention reaches a saturation value after a few hours of heating. Very recently, experimental evidence was obtained which indicates that the ammonium group in a compound can influence the course of thermal and radiation annealing[1]. Potassium and ammonium dichromate were irradiated with thermal neutrons under the same conditions and their behaviour in thermal annealing was compared. Retention of potassium salt for various temperatures was as expected, while the retention of ammonium salt passed through a maximum above 170° C. This suggests that a reduction process is taking place at the same time as thermal annealing. Similar results have been obtained for the radiation annealing process.

Experiments with ammonium sulphate were carried out in order to see if this anomaly is also common to other ammonium compounds. The sample of 'Analar' grade ammonium sulphates was irradiated in a polythene tube in the thermal column of the *Bepo* reactor at Harwell for four weeks at a neutron flux of 10^{10} n./cm.^{2}/sec. (pile factor 0·1). The temperature during the irradiation was below 50° C. Together with sulphur-35 formed by the nuclear reaction $^{34}S(n,\gamma)^{35}S$, $^{32}P[^{32}S(n,p)^{32}P]$ and $^{33}P[^{33}S(n,p)^{33}P]$ were also produced. Phosphorus-32 and -33 were subjected to separate investigations.

Under the conditions of irradiation used in these experiments sulphur-35 is distributed into several fragments because of recoil. It was found that about 60 per cent of the total sulphur activity was in the form of sulphate, the rest being present as sulphite. It is possible that other fragments of sulphur could be formed as intermediates which, however, are readily transformed into sulphate and sulphite under the conditions of analysis. The samples were dissolved in oxygen-free, boiling, diluted hydrochloric acid together with sulphate and sulphite carrier and phosphate hold-back carrier. Thereby sulphite and other volatile sulphur compounds were immediately separated as gases. These were removed by a stream of nitrogen and absorbed under oxidation in strongly alkaline hydrogen peroxide solution. This part of the sulphur activity as well as the remaining sulphate activity were precipitated as barium sulphate and measured with a suitable end-window Geiger counter. All the necessary corrections have been made.

The thermal annealing of the irradiated ammonium sulphate was carried out in an oven at various temperatures and various heating periods in the presence of air. The heated samples were analysed as stated here. The retention at 100° ± 0·5° C. was plotted against heating time and found to follow a typical annealing process curve. However, at temperatures above 150° C., the curve passed through a maximum. This anomaly was most conspicuous at 180° ± 0·5° C., as shown in Fig. 1. A certain radiation annealing is assumed to occur also during the pile radiation.

It is supposed that free radicals are formed in the target under the influence of the γ-radiation in the pile, probably in a relatively large 'steady-state'

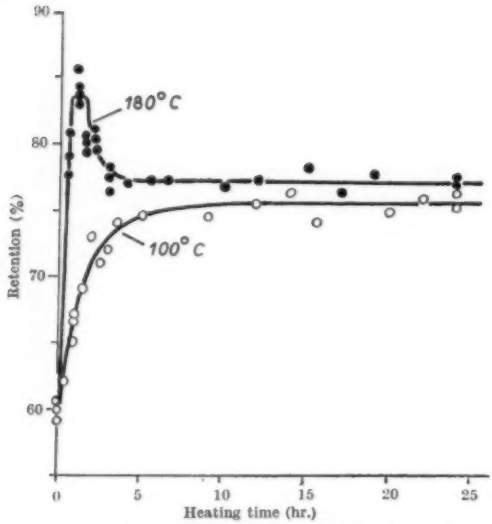

Fig. 1. Thermal annealing at 100° and 180° C. of ammonium sulphate irradiated in *BEPO*, Harwell, for 4 weeks

concentration, which are available for reaction with the recoil-atoms. The ammonium group can be transformed under these conditions into certain fragments, which could give rise to formation of reducing compounds, for example, hydrazine. This could either occur during the irradiation in the pile or later in the thermal annealing process. All these products formed from the ammonium group can act as reducing agents during the annealing process, although the reducing effect becomes clear only at higher temperatures.

The reformed sulphate arises chiefly during the process of thermal annealing directly from the reduced sulphate fragments. Another contribution could also be the oxidation of some recoil fragments which form sulphate on dissolving. Consequently, two counteracting processes are taking place in the irradiated ammonium sulphate during the period of heating at temperatures greater than 150° C., namely, thermal annealing and reduction.

A full report on the role of the ammonium group in thermal annealing will be published in the *Mitteilungen des Instituts für Radiumforschung in Vienna* (Proceedings of the Austrian Academy of Sciences).

We are indebted to Prof. B. Karlik for her interest in this work. One of us (M. N.) wishes to thank the Bundesministerium für Unterricht in Austria for a fellowship.

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Radiolysis of Liquid Acetone: Effect of Dose-rate and Linear Energy Transfer

The effect of the linear energy transfer parameter of the radiation and of dose-rate on the radiolysis of aqueous solutions can be understood in terms of interactions between spurs along a track and between spurs on adjacent tracks[1]. In contrast the radiolysis of organic liquids shows: (1) no effect of linear energy transfer on pure alkane liquids[2], although a slight effect has been found with solutions containing iodine[3], and a pronounced effect with aromatic liquids[4] (see also ref. 5); (2) effects at much lower values of dose-rate than in the case of aqueous solutions[6].

In the course of a more general study of the radiolysis of liquid acetone I have found that the yields of products are affected both by the linear energy transfer of the radiation and also the dose-rate. The G value for carbon monoxide production was found to decrease with increasing dose-rate: this is somewhat surprising, but may be due to scavenging of radicals by carbon monoxide, for which there is some evidence[7]. Since this trend would imply that less methyl radicals are available for reaction at high dose-rates than at low ones, I have normalized the yields of methane, ethane and methyl ethyl ketone (these are thought to be the only products arising from reactions of methyl radicals) to the yield of carbon monoxide and plotted them against dose-rate in Fig. 1. The radiation used was γ-rays from a cobalt-60 source and 1-MV. electrons from a Van de Graaff machine. All runs were carried out at 20° C. and in a dose-range where it had previously been shown that G values were independent of absorbed dose.

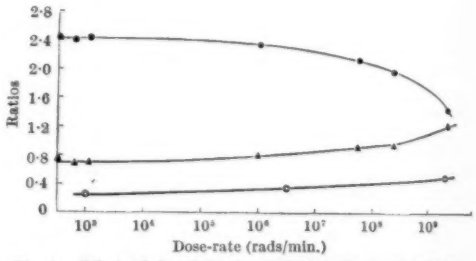

Fig. 1. Effect of dose-rate on product ratios. ●, CH_4/CO; ▲, $(C_2H_6)^{1/2}/CO$; ○, $CH_3CH_2CO\ CH_3/CO$

It is known that more than three-quarters of the yield of methane in the radiolysis of pure liquid acetone at low dose-rates is derived from the reaction of a thermal methyl radical[8]:

$$CH_3. + CH_3.CO.CH_3 \longrightarrow CH_4 + CH_2.COCH_3 \quad (1)$$

and it is probable that ethane and methyl ethyl ketone arise from the reactions:

$$CH_3. + CH_3. \longrightarrow C_2H_6 \quad (2)$$

$$CH_3. + CH_3.CO.CH_3 \longrightarrow CH_3.CH_2.CO.CH_3 \quad (3)$$

The variation of the product ratios with dose-rate shown in Fig. 1 can then be explained in terms of the competing reactions (1), (2) and (3) and the overlapping of 'diffused' spurs at higher dose-rates. The dose-rate at which such an effect starts to occur is mainly determined by the efficiency of reaction (1) in removing methyl radicals. Since reaction (1) is very slow compared with 'normal' scavenging reactions, the effect on the yields of products occurs at much lower dose-rates than in aqueous solutions[9]. Knowing the rate of reaction (1) one can calculate the dose-rate above which combination of methyl (and other) radicals diffusing from spurs on adjacent tracks might be expected to occur. This gives the same value (within the limits of the calculation) as that shown in Fig. 1. The decrement in CH_4/CO on going from low to high dose-rate ($= 0{\cdot}97$) is greater than the increment in $(2C_2H_6 + CH_3\,CO.CH_2.\,CH_3)/CO = (0{\cdot}71)$: this one

might anticipate, since we have no experimental measure of the reaction :

$$CH_3 + .CO.CH_3 \longrightarrow CH_3.CO.CH_3 \qquad (4)$$

which should be more important at higher dose-rates.

Table 1 contains G values of some products as a function of dose-rate (cobalt-60γ and electron irradiations) and of linear energy transfer of the radiation at the same dose-rate (electron and proton irradiations). Comparison of the results of γ- and proton-irradiation illustrates more markedly the effect of dose-rate and linear energy transfer on the products of the radiolysis of acetone, which can be explained as an enhancement of reactions (2) and (3) at the expense of reaction (1), occurring between radicals from spurs on adjacent tracks and between radicals from spurs on the same track. It will be noted that G_{CO} appears to be the same for the γ- and proton-irradiations and that G_{CH_4} (total) for γ-irradiation is equal to $2{\cdot}47 \pm 0{\cdot}18$, compared with G_{CH_4} (total) equal to $2{\cdot}24 \pm 0{\cdot}25$ for proton irradiations: the difference, if significant, may again be a measure of reaction (4).

Table 1. Product Yields as a Function of Dose-Rate and Linear Energy Transfer

Radiation dose-rate in rads/min.	Cobalt-60γ $3{\cdot}3 \times 10^3$	1-MV. electrons $5{\cdot}4 \times 10^7$	2-MV. protons $5{\cdot}4 \times 10^7$
G_{H_2}	$0{\cdot}44 \pm 0{\cdot}09$	$0{\cdot}47 \pm 0{\cdot}10$	$0{\cdot}92 \pm 0{\cdot}06$
G_{CO}	$0{\cdot}76 \pm 0{\cdot}01$	$0{\cdot}47$	$0{\cdot}73 \pm 0{\cdot}08$
G_{CH_4}	$1{\cdot}85 \pm 0{\cdot}05$	$0{\cdot}78$	$0{\cdot}59 \pm 0{\cdot}14$
$G_{C_2H_6}$	$0{\cdot}25 \pm 0{\cdot}04$	$0{\cdot}25 \pm 0{\cdot}04$	$0{\cdot}56 \pm 0{\cdot}03$
G_{MEK}	$0{\cdot}21 \pm 0{\cdot}01$	$0{\cdot}20 \pm 0{\cdot}01$	$0{\cdot}53 \pm 0{\cdot}05$ *

* Dose-rate = $9{\cdot}6 \times 10^5$ rads/min.

These experiments together with scavenging experiments allow some speculation to be made about the mode of formation of some of the products. It has been suggested[8] that ethane is formed in the low dose-rate irradiation entirely from radical–radical reactions in the spur, and this is supported by the present work. The suggestion[7,8] that hydrogen formation arises from an abstraction reaction of hot hydrogen atoms appears in conflict with the dependence of the yield on linear energy transfer : the latter indicates a hydrogen atom recombination process in the spur. Scavenging experiments (with iodine) showed that at low dose-rates at least half and probably appreciably more of the yield of methyl ethyl ketone was unaffected: this product is therefore likely to be formed in radical–radical reactions in spurs also.

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CHEMISTRY

Fundamental Relaxation of Classical Oscillators

The relaxation of systems of gaseous oscillators and the related process of non-equilibrium chemical reaction have recently received detailed consideration. However, while satisfactory formal theories have been presented both for the quantum[1–4] and classical[5,6] cases, little progress has been made in formulating the detailed transition probabilities which control the time evolution of these systems, and little can be expected in view of the extraordinarily complex nature of the collision process.

The present purpose is to direct attention to an important limiting case in the classical theory. Vibrational energy alone is considered and is assumed to be conserved during all collisions. If now the precise details of the collisional act are ignored and it is assumed instead that the total energy is redistributed 'statistically' in every collision, then the transition probability distribution (transition kernel) governing the exchange between single harmonic oscillators and a heat bath consisting likewise of single oscillators may easily be shown to be the following:

$$k(E_1,E_2) = (Z/kT)\exp(E_1/kT)\,\mathrm{ei}[\max(E_1,E_2)/kT] \qquad (1)$$

Here Z is the collision number per molecule per unit time, $\max(E_1,E_2)$ is the maximum of a given initial and final energy, kT has the usual significance, T being the heat-bath temperature, and $\mathrm{ei}(x) = \int_x^\infty (1/s)e^{-s}ds$ is the exponential integral. ($k(E_1,E_2)dE_2/Z$ is the probability that an oscillator initially with energy E_1 will undergo a transition to dE_2 at E_2 on collision with a heat-bath oscillator.)

This result follows immediately on considering the probabilities that: (1) an oscillator with energy E_1 will form a collision complex with specified total energy; (2) the complex will divide to leave the oscillator of interest with energy in dE_2 at E_2, all distributions assumed equally probable, then finally integrating over all possible collisions and multiplying by the collision number.

It will be seen that $k(E_1,E_2)$ has a singularity at $k(0,0)$ while from a given energy all down-transitions are equally probable.

The relaxation process is described by a time-dependent distribution function $c(E,t)$ which is the solution of the following continuity equation[6]:

$$\left(\frac{\partial}{\partial t} + Z\right)c(E,t) = \int_0^\infty k(\xi,E)c(\xi,t)d\xi \qquad (2)$$

Now it is known that the transition kernel (1), with a symmetrizing factor, is essentially the Green's function corresponding to the Laguerre polynomials, and the solution of the above equation with this kernel is in fact:

$$c(E,t) = (1/kT)\exp(-E/kT)\left[1 + \sum_{k=1}^{\infty} c_k L_k(E/kT)\exp\left(-\left(\frac{k}{k+1}\right)Zt\right)\right] \qquad (3)$$

where c_k is determined by the initial distribution $c(E,0)$ through the orthogonality property of the polynomials:

$$c_k = \int_0^\infty c(E,0) L_k(E/kT)\, dE \qquad (4)$$

In the special case where the initial distribution is itself a Boltzmann one, though at a different temperature from the heat bath, it can be shown that:

$$c_k = (1 - T/T_0)^k \qquad (5)$$

T, T_0 being the temperatures of heat bath and initial distribution respectively.

The importance of the transition kernel derived here is that it expresses what, within the scope of the model, is the most efficient possible coupling between heat bath and system.

An extended account of the above approach, together with its application to the theory of chemical reaction, is in preparation.

I am indebted to the Office of Naval Research, Washington, and the Department of Scientific and Industrial Research, London, for financial assistance at different stages of this work.

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Thermal Decomposition of Ammonium Perchlorate in Presence of Zinc Oxide

The decomposition of ammonium perchlorate has been examined by Bircumshaw and Newman[1], and Galwey and Jacobs[2], respectively. They have observed that the decomposition of the salt occurs according to two different mechanisms. At lower temperature (between 200° and 280° C.), when only about 30 per cent of the substance decomposes, the electron transfer mechanism dominates, the energy of activation is 32 kcal., and at higher temperature (between 350° and 400° C.) the proton transfer mechanism plays a decisive part (E = 39·5 cal.).

Recently, we have investigated the influence of several metal oxides on thermal decomposition of different solids (potassium chlorate, potassium perchlorate, etc.)[3]. The most effective catalyst for both reactions was nickel oxide, which not only increased the reaction-rate but also lowered the energy of activation of the process. According to earlier observations the rate of decomposition of ammonium perchlorate is influenced by metal oxides[4], especially by manganese dioxide. In our experiments with ammonium perchlorate from among nickel, zinc, ferric and cadmium oxides it was especially the zinc oxide which was outstanding.

As a rule, the experiments were carried out both in air and *in vacuo*, and the reaction-rate was followed by determining the amount of gas evolved. The decomposition of ammonium perchlorate between 200° and 300° C., in absence of a catalyst, occurred to the extent of 30 per cent. Kinetical results were evaluated by the Prout–Tompkins equation, the unimolecular decay and Avrami–Erofeev's equations respectively. The activation energies calculated from the dependence of rate constants on temperature agree generally fairly well with those calculated by the authors referred to.

The decomposition of ammonium perchlorate was effectively catalysed by zinc oxide and then decomposition occurred at 240° C. to the extent of 75–85 per cent. However, as in our earlier experiments the rate of reaction decreased in each case with the decrease of the amount of the catalyst[3]; now the rate scarcely depended on the amount of zinc oxide and a considerable catalytic effect was observed even in the presence of about 0·1 per cent of the oxide. The contracting-cube formula was found to be the best to evaluate the rate constants in the catalysed decompositions. Some data on the energy of activation found at different ammonium perchlorate and zinc oxide mixtures are given in Table 1.

Table 1

NH_4ClO_4 : ZnO (mole ratio)	Activation energy *
50 : 1	29·5 kcal.
16 : 1	33·3 ,,
1 : 1	33·5 ,,

* Rate constants were calculated from the equation $1-(1-\alpha)^{1/3} = Kt$.

These values are approaching 32 kcal., which corresponds to the electron transfer mechanism, indicating that in the temperature-range investigated the electron mechanism plays a part even in the presence of zinc oxide. As is well known, zinc oxide belongs to the *n*-type group of semiconductors. Its electric properties can be effectively influenced by doping with small amounts of foreign ions of different valence. Thus we have studied the effect of changes in the electric conductivity of zinc oxide on the catalysed decomposition of ammonium perchlorate. According to our measurements the rate of reaction was accelerated by increasing the conductivity of zinc oxide (building-in of 1 per cent of aluminium oxide) and it was lowered by decreasing the conductivity (building-in of 1 per cent of lithium oxide); and it largely lengthened the induction period. On the contrary, activation energies practically do not change on doping with zinc oxide.

While the catalysed decomposition of ammonium perchlorate could at 240° C. be followed easily, above this temperature the course of kinetic curves changed, and already in presence of 0·1 per cent zinc oxide, after an induction period, the substance exploded. The explosion occurred very sharply at mole fraction of 300 : 1–10 : 1 ammonium perchlorate–zinc oxide and it was accompanied by a luminous phenomenon. In presence of zinc oxide applied in equal mole ratio the decomposition of ammonium perchlorate similarly resulted in an explosion, but not so violently as in the case of mixtures containing a small quantity of zinc oxide. By further increasing the concentration of zinc oxide, instead of explosion, degeneration occurred. From dependence on temperature of the times until explosion we obtained the energies of activation, given in Table 2, which are practically independent of the amount of zinc oxide between 1 : 1 and 100 : 1 mole ratio of ammonium perchlorate–zinc oxide mixtures.

Table 2

NH_4ClO_4 : ZnO (mole ratio)	Activation energy
100 : 1	29·5 kcal.
50 : 1	28·0 ,,
25 : 1	27·0 ,,
10 : 1	27·6 ,,

According to Galwey and Jacob's measurements[2] the explosion of ammonium perchlorate at 1 atm. pressure occurs only at temperatures above 440° C. From the dependence on temperature of the induction period these authors obtained almost identical value for the activation energy (41·5 kcal.) as in the decomposition of ammonium perchlorate at high

temperatures: from what they stated it appears that the proton transfer mechanism plays a part in the explosion. In our experiments the explosion temperature was lowered almost to 200° C. by a very small amount of zinc oxide (0·2 per cent). The values of activation energy found by us are considerably lower than those obtained by Galwey and Jacobs and well approach those corresponding to the electron transfer mechanism and determined at a lower temperature.

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Separation, Identification and Determination of the Fatty Acids of Montan Wax

Crude montan wax is essentially a mixture of the esters of long-chain carboxylic acids, with free acids, resin and asphalt[1]. Much of the work devoted to a study of the wax acids prior to 1930 is clearly unreliable. In that year, using micro-distillation and X-ray analysis, Holde and Bleyberg[2] isolated and identified a normal C_{28} acid, and indicated the presence of a normal C_{26} acid. Further progress was made by Fuch and Dieberg[3] using absorption and elution chromatography and infra-red spectroscopy. They succeeded in isolating and identifying all the normal straight-chain carboxylic acids with even carbon numbers from C_{22} to C_{34}.

Using gas-chromatography, we have succeeded in resolving the methyl esters of carboxylic acids from a saponified bleached montan wax. A total of 11 components were separated, and identified as the esters of the normal straight-chain acids with both even and odd carbon numbers from C_{22} to C_{32}, inclusive. The C_{24} acid identified by Fuch and Dieberg was not detected.

This separation was achieved using a 'Shandon' gas-chromatograph and a flame-ionization detector. The column originally used was 4 ft. in length and contained 100–200 mesh 'Celite' with 10 per cent polyethyleneglycol adipate as stationary phase. More recently the columns have been reduced in length and the packing has included also 1 per cent of orthophosphoric acid[4].

The identifications were made using the well-known relation of the logarithms of retention times with carbon numbers for each homologous series. From the work of Fuch and Dieberg, establishing the presence of the normal straight-chain carboxylic acids C_{22} to C_{34}, it should be possible to assign carbon numbers to the retention times recorded, provided that one or preferably two reference retention times are known. Such reference times were obtained from stearic and palmitic acids present in a commercial sample of stearic acid. From these it was possible to identify not only the C_{22} to C_{32} even-numbered acids noted by Fuch and Dieberg but also the remaining 5 components, as the intervening odd-numbered acids.

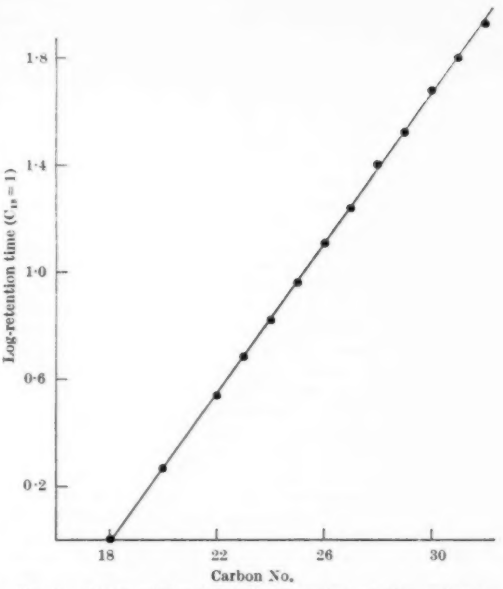

Fig. 1. Linear relationship between carbon number and log-retention time

The validity of this identification was confirmed by an examination of arachidic C_{20} and behenic C_{22} methyl esters. The linear relation of retention for all the methyl esters recorded is shown in Fig. 1 (methyl stearate = 1).

It has been possible to derive an approximate composition of the mixture of esters by measuring the areas below the chromatogram, when the following was obtained:

	C_{16}	C_{18}	C_{20}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}
Per cent less than 0·05 each				0·25	0·25	7·5	2·0	22·0	3·5	27·5	3·0	24·0	2·0	8·0

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BIOCHEMISTRY

Distribution of Potassium in Baker's Yeast

Baker's yeast in the resting state normally contains a relatively large amount of potassium. In addition, it has been shown by a number of workers that during active metabolism (aerobic or anaerobic utilization of glucose[1] or aerobic respiration of certain other substrates[2]) considerable net absorption of potassium can occur even though the concentration of potassium in the external medium may be very much less than that inside the cells. Accumulation of potassium, and of other cations, by yeast involves active transport of the ions across the cell membrane[3], the inward movement of ions being mediated by

relatively specific carrier mechanisms[4] therein. Many facts require this interpretation and have been stated in detail elsewhere.

The results of an investigation recently carried out in this laboratory[5] showed that the presence of relatively large amounts of bound potassium within the yeast cell is very unlikely. In these experiments the total intracellular concentration of free solutes in washed and centrifuged yeast, which had previously been subjected to a repeated process of freezing in liquid oxygen and thawing, was determined by a microcryoscopic method, a mean value, for resting yeast, of 0·59 *M* being obtained. This value was in close agreement with that calculated from the known concentrations, determined by chemical analysis, of low-molecular weight substances, including potassium, in the same strain of yeast (0·60 *M*). In dialysis experiments in which the fluid material obtained by alternate freezing in liquid oxygen and thawing of the centrifuged cells was allowed to come to equilibrium with a relatively small volume of distilled water, it was found that the total intracellular concentration calculated from the freezing-point depression of the dialysate was in good agreement with that obtained from direct measurements on the frozen and thawed yeast. From these results it seemed possible that a determination of the proportion of the total potassium in yeast appearing in such dialysates might yield some information about the extent to which this ion is bound in the cells.

A series of experiments was carried out in which fresh yeast was twice washed in de-ionized water and centrifuged, the second centrifuging being continued for 20 min. at 3,000 r.p.m. The packed cells from this second centrifuging (which were contained in stoppered plastic centrifuge tubes) were then frozen in liquid oxygen and allowed to thaw, this procedure being repeated twice. 2-ml. samples of the liquid material from the freezing and thawing process were dialysed overnight in the cold against an equal volume of de-ionized water and the potassium content of the dialysate determined. This was compared with the total potassium estimated directly on a sample of the frozen and thawed yeast. In a group of nine experiments of this kind, the total potassium ranged from 102·5 to 130·0 m.equiv./kgm. washed and centrifuged yeast, the mean value being 119·0 m.equiv./kgm. The potassium concentration in the dialysates ranged from 94·0 to 125·0 m.equiv./kgm. with a mean of 109·0 m.equiv./kgm.

These results indicated that by far the greater proportion of the total potassium content of yeast is free to diffuse out of the cells once the membrane has been broken by suitable means, and that the amount bound to high-molecular weight cytoplasmic constituents is relatively small. This small amount of bound potassium, amounting in the average to about 8 per cent of the total, appears to be quite firmly held. In experiments in which the solid residue from the dialysis process was washed in about 50 times its own volume of de-ionized water it was found that, after three such washings, some 50–60 per cent of it still remained bound to the solid cell debris. The unlikelihood of relatively large amounts of potassium, previously bound, being released during dialysis is indicated by the close agreement observed[5] between the total intracellular concentration determined from the freezing-point depression of such dialysates and that of the frozen and thawed yeast.

Further experiments were concerned with an attempt to determine the distribution between these two fractions of the extra potassium entering the cells during fermentation under a variety of conditions. In these experiments the yeast, after fermentation, was twice washed in de-ionized water as before, frozen in liquid oxygen and thawed and the total and 'dialysable' potassium estimated as described above. In each case the results were compared with those obtained from a sample of the same yeast in the resting state. These results are summarized in Table 1.

From Table 1 it is seen that, with the exception of the long-period fermentation in potassium citrate (sample 4), virtually all the extra potassium accumulated during fermentation appears in the freely dialysable fraction. In the case of samples 1 and 2, where the external potassium concentration is relatively low, the results shown in Table 1 would appear to indicate the accumulation of free potassium against a high concentration gradient.

Table 1. Accumulation of Potassium by Yeast fermented under Various Conditions

Sample	Total potassium		Potassium accumulated	Potassium in dialysate		Potassium accumulated
	Resting	Fermented		Resting	Fermented	
1	127·5	145·5	18·0	121·0	139·5	18·5
2	118·0	129·0	11·0	114·0	122·5	8·5
3	123·0	150·0	27·0	111·5	137·0	25·5
4	111·0	140·0	29·0	98·0	112·0	14·0

In all cases, 1 part by weight of washed and centrifuged yeast was suspended, with aeration, in 20 vol. of fluid containing 5 per cent glucose. Other conditions were as follows: Sample 1: fermentation for 1 hr. in the presence of 10 m.moles/litre of potassium chloride plus collidine/hydrochloric acid buffer. Final *p*H of suspensions, 4–4·5. Sample 2: conditions as for 1, but with 2 m.moles/litre external potassium chloride. Sample 3: fermentation for 45 min. in the presence of 200 m.moles potassium acetate. Sample 4: fermentation for 4 hr. in potassium citrate (200 m.equiv. potassium ions/litre).

All values are in m.equiv./kgm. washed and centrifuged yeast. Each sample represents the mean of two experiments with duplicate estimations in each experiment.

Table 2. Distribution of Sodium and Potassium in Sodium-rich Yeast

Yeast	Potassium (m.equiv./kgm.)		Sodium (m.equiv./kgm.)	
	Total	In dialysate	Total	In dialysate
Resting	112·0	97·0	—	—
Sodium-rich	55·0	34·0	80·0	80·0

Results give the mean for three experiments each with duplicate estimations.

Conway and Moore[6] have shown that when yeast is fermented in 5 per cent glucose with the addition of sodium citrate, there is a marked accumulation of sodium, potassium being lost. In Table 2 is shown the results of an investigation by the technique outlined here of the distribution of sodium and potassium in a sodium yeast prepared by fermenting fresh yeast for two successive 2-hr. periods in 20 volumes of a sodium citrate-glucose medium containing 200 m.equiv. sodium ions per litre. From Table 2 it appears that virtually all the sodium accumulated is in a freely diffusible form. In these experiments there appeared to be some increase in the amount of bound potassium in the cells.

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Comparison of the Mitochondria of the Small Intestine of Vertebrates

MITOCHONDRIA of the intestinal mucosa of the following vertebrates have been examined by electron microscopy: the rat (*Rattus norvegicus*), guinea pig (*Cavia percellus*), chicken (*Gallus domesticus*), lizard (*Tiliqua rugosa*), toad (*Bufo marinus*) and carp (*Carassius auratus*) (Fig. 1). The mitochondria in the intestinal cells of these species showed no major differences in structure from the other mitochondria that have already been described[1,2]. The intestinal mitochondria from all these species swelled when they were isolated by differential centrifugation in 0·25 *M* sucrose (Fig. 2), but all except those from the rat exhibited considerable succinoxidase activity as measured manometrically (Table 1). The oxygen uptake of homogenates of the intestinal mucosa of rats was also low and variable compared with that of homogenates of intestinal mucosa prepared from guinea pigs. In contrast, the oxygen uptake (per mgm. dry weight) of whole intestinal cells of both the rat and the guinea pig was similar.

Homogenates of the intestinal mucosa of rats were found to inhibit the oxygen uptake of homogenates of the intestinal mucosa of guinea pigs. The inhibitor present in the rat intestinal homogenate was not destroyed by heating for 3 min. in a boiling water bath, or by adding hydrochloric acid to a final concentration of 2 *N*. The inhibitor was not dialysable, but it could be extracted with iso-octane. The iso-octane extract contained a substance that absorbed light strongly at wave-lengths below 250 mμ. This suggested the presence of long-chain unsaturated fatty acids. Chromatographic examination of the extract showed the presence of oleic acid and a smaller amount of linoleic acid. Small quantities of oleic acid

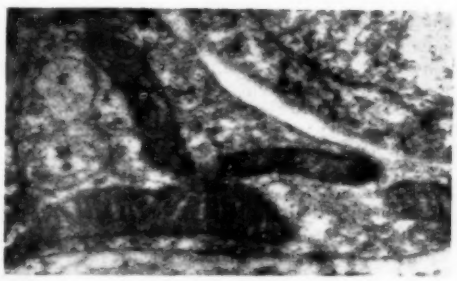

Fig. 1. Electron micrograph of a section of a cell of the intestinal mucosa of *Carassius auratus* showing the mitochondrial structure. × 35,000

Fig. 2. Electron micrograph of a section of mitochondria isolated from the intestinal mucosa of *Carassius auratus*. × 37,500

Table 1. SUCCINOXIDASE ACTIVITY EXPRESSED AS THE $Q_{O_2}N$ OF MITOCHONDRIA ISOLATED FROM THE INTESTINAL MUCOSA OF VARIOUS VERTEBRATES

Animal	$Q_{O_2}N$	Standard error
Rat	< 15	—
Guinea pig	420	13
Chicken	526	13
Lizard	467	13
Toad	212	25
Carp	356	13

(≏ 0·005 ml./mgm. mitochondrial N), or of the material recovered from the iso-octane extract, inhibited by 95 per cent the succinoxidase activity of mitochondria isolated from guinea pigs. The inhibitory effect of oleic acid on a succinoxidase preparation from ox-heart has previously been reported[3]. Nakamura *et al.*[4] also ascribed the low succinoxidase activity of rat intestinal mucosa to the presence of fatty acids. That mitochondria from the intestine of the rat do contain succinic dehydrogenase can be shown cytochemically. When these mitochondria are incubated with 2 : 3 : 5 triphenyl tetrazolium chloride in the presence of succinate, deposits of formazan can be seen. No reduction of the dye occurs in the absence of succinate. As cytochrome oxidase has been shown[5] to donate electrons to triphenyl tetrazolium chloride, it is suggested that the inhibitor of succinoxidase released on the rupture of rat intestinal cells acts between cytochrome oxidase and oxygen. Some evidence to confirm this has been obtained. The cytochrome oxidase activity (measured manometrically) of mitochondria isolated from the intestinal mucosa of the rat is less than one-tenth of that of intestinal mitochondria isolated from the guinea pig.

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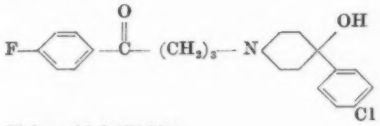
Combustion Analysis of Paper Chromatographic Spots

THE quantitative determination of paper chromatographic spots is usually carried out by using a variety of photometric techniques. In many instances, however, the known methods lack specificity, reliability and sensitivity.



Haloperidol (*R*1625)

For haloperidol[1–3], a new potent neuroleptic agent recently developed in this Laboratory, a satisfactory method of photometric analysis, suitable for the analysis of minute amounts in chromatographic spots, has not yet been found. I have therefore developed an alternative method, based on the quantitative micro-analysis of the organic fluorine content of the spots.

Fluorine (μgm.)			
Actual	Estimated		
0·40	0·25	0·45	0·50
0·80	0·77	0·72	0·60
1·20	1·28	1·14	1·08
1·60	1·68	1·44	1·60
2·00	2·05	2·20	1·70

After one-dimensional chromatographic separation (using methanol, amyl alcohol, benzene, water, 40/20/40/5) on buffered[4] Whatman No. 1 paper, of a solution containing 8–40 μgm. of haloperidol, the haloperidol spots are located by inspection under an ultra-violet light source. The fluorescent area is then cut out and burned in a modified Schöniger oxygen flask[5–8], 150 ml. in volume and with a small valve constructed at the bottom of the flask. The combustion products are absorbed in 3 ml. of water and the inorganic fluoride determined by the colorimetric method of Singer and Armstrong[9]. In a series of 15 experiments, the estimated haloperidol content of the spots varied between 85 and 110 per cent of the actual amounts (8–40 μgm.) which were put on the paper chromatogram.

This general method should be applicable to the quantitative analysis of paper chromatographic and paper electrophoretic spots of compounds containing other halogens, sulphur, phosphorus, arsenic, boron, cadmium, mercury and zinc[10].

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Inactivation of *A*-Methopterin by the Acetylating Enzyme System from Pigeon Liver

A-METHOPTERIN (4-amino-N[10]-methyl pteroyl glutamic acid) inhibits the growth of certain tumours[1] as well as of many folic acid dependent micro-organisms[2]. It is a powerful inhibitor of enzyme systems reducing folic acid to tetrahydrofolic acid[3]. Recent investigations[4,5] have shown that *A*-methopterin inhibits acetylation of aromatic amines. Small quantities of *A*-methopterin have been found to decrease considerably the urinary excretion in mice of the acetylated aromatic amine formed from injected folic acid (unpublished results). Evidence presented here shows that *A*-methopterin is inactivated by the acetylating enzyme system of pigeon liver.

Crude enzyme preparations employed in the present work consisted of 5 per cent extracts of pigeon liver acetone powder in 0·2 *M* sodium fluoride. *A*-methopterin was assayed by the inhibition analysis using *S. faecalis* as the test organism[6]. Initial experiments showed that the assay system was most sensitive in the presence of 0·8 mμgm. of folic acid per ml. The dilutions of the test material were made so that the final concentration of *A*-methopterin in the assay system was 0·5–0·8 mμgm. per ml. Each determination was done in duplicate at 3 different levels of dilution.

Table 1. Effect of Omission of Components of the Acetylating System on the Inactivation of *A*-Methopterin

Exp. No.	System	Aliquot (ml.)	Time of incubation (min.)	Growth of *S. faecalis*: Klett units 660 mμ	Growth of *S. faecalis*: Expressed as percentage of control in absence of *A*-methopterin
1	Complete system (Crude enzyme extract)	0·6	0	76	42·0
	(Crude enzyme extract)	0·6	180	118	65·6
	Autoclaved system*	0·6	180	87	48·0
2	Complete system (Fractions *A*-40 + *A*-60)	0·6	0	90	45·5
	(Fractions *A*-40 + *A*-60)	0·6	180	132	65·7
3	Fraction *A*-40 omitted	0·6	0	78	36·4
	Fraction *A*-40 omitted	0·6	180	76	35·0
4	Fraction *A*-60 omitted	0·6	0	70	35·0
	Fraction *A*-60 omitted	0·6	180	66	33·0
5	Acetate omitted	0·6	0	91	45·5
	Acetate omitted	0·6	180	91	45·5
6	CoA omitted	0·6	0	35	17·8
	CoA omitted	0·6	180	33	17·0
7	ATP omitted	0·6	0	92	48·0
	ATP omitted	0·6	180	84	44·5

Incubation system: Na_2HPO_4, 0·02 *M*; $NaHCO_3$, 0·028 *M*; acetate, 0·02 *M*; ATP, 0·004 *M*; *A*-methopterin, 13·2 μgm.; CoA, 12·5 μgm.; Mg^{++}, 0·0055 *M*; cysteine, 0·005 *M*; crude enzyme extract (0·5 ml.) or fraction *A*-40 and *A*-60, 0·4 ml. each. Volume, 3·0 ml. pH 7·0; temperature, 37° C.
* Autoclaved at 10 lb. pressure for 10 min.

Table 2. Effect of *p*-Aminobenzoic Acid on the Inactivation of *A*-Methopterin

Exp. No.	System	Aliquot (ml.)	Time of incubation (min.)	Growth of *S. faecalis*: Klett units 660 mμ	Growth of *S. faecalis*: Expressed as percentage of control in absence of *A*-methopterin
1	Crude enzyme system	0·4	0	75·0	43·0
	Crude enzyme system	0·4	180	125·0	71·8
2	Crude enzyme system + PABA (13·2 μgm.)	0·4	0	86·0	49·7
	+ PABA (13·2 μgm.)	0·4	180	107·0	61·5

Incubation system same as in Table 1.

The inactivation of *A*-methopterin in the test system occurred rapidly. Values more consistent with the quantity of added *A*-methopterin were, however, obtained by autoclaving the zero-time samples at 10 lb. for 10 min. prior to assay. The sterilization of the test samples was carried out by filtration through sintered-glass bacteriological filters.

Results given in Table 1 show that incubation of *A*-methopterin for 180 min. with the complete acetylating system from pigeon liver leads to more than 40 per cent decrease in the activity of *A*-methopterin as a growth inhibitor of *S. faecalis*. The fact that the original inhibitory activity of *A*-methopterin is recovered by autoclaving the incubated mixture indicates the formation of a less active or an inactive derivative of *A*-methopterin which is unstable to high temperatures.

The effect of omission of various components of the acetylating system on the inactivation of *A*-methopterin was investigated using pigeon liver extracts prepared according to Chou and Lipmann[7]. In these preparations, fraction *A*-40 activated acetate to form acetyl-coenzyme A and fraction *A*-60 catalysed the transfer of the acetyl group to the acceptor amine.

The results (Table 1) demonstrate that the inhibitory activity of *A*-methopterin is decreased only in the presence of the enzyme system containing all the co-factors (adenosine triphosphate, coenzyme A and acetate) required for the acetylation of aromatic amines. By extensive dialysis, it was possible to demonstrate the requirement for acetate. The inactivation of *A*-methopterin did not occur in absence of any one of the active protein fractions. This excludes

the possibility of inactivation due to non-specific adsorption on proteins. *p*-Aminobenzoic acid (PABA), which can be acetylated by the pigeon liver enzyme system and so may be expected to compete with the acetylation of other amines, was consistently found to diminish the inactivation of *A*-methopterin (Table 2). The quantity of extra PABA added in these experiments did not affect the growth in the control system. These observations, and the fact that the *A*-methopterin molecule contains the necessary structural features, suggest that the inactivation of *A*-methopterin is brought about through the formation of acetyl derivatives which are less active or completely inactive as inhibitors for the growth of *S. faecalis*. Aminopterin is also inactivated by a similar enzyme system, but to a lesser extent than *A*-methopterin. Earlier work of Jacobson[8] on the effect of aminopterin on cell division have indicated the formation of an inactive derivative from aminopterin on incubation with various animal tissues, including leukæmic cells. Fountain *et al.*[9] have reported that the concentration of *A*-methopterin detected in liver and kidney of mice injected with *A*-methopterin increased several fold when the samples were boiled before assay. Since both these organs contain the acetylating enzymes, the increased activity produced on boiling the tissue could have resulted from breakdown of acetyl derivatives of the type which the present work has shown to be unstable to high temperatures.

A-methopterin was found to inhibit the acetylation of sulphanilamide in pigeon liver preparations but had no effect on acetyl-CoA formation. Similar results have been obtained also by Johnson *et al.*[4]. Apparently *A*-methopterin inhibits the acetylation of aromatic amines by interference with the transferase component of the overall system. The removal of acetyl groups for the acetylation of *A*-methopterin as inferred from the present findings could explain the type of inhibition produced by *A*-methopterin on biological acetylations.

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Improved Benzidine Sugar Reagent for Paper Chromatograms

Benzidine has been found to be a useful reagent for sugar detection on paper chromatograms[1,2] and for quantitative determination in test-tubes[4]. Horrocks[3] first found that benzidine–acetic acid detected reducing aldoses on paper chromatograms, but ketoses were detected only at high concentration. Bacon and Edelman[1] added trichloracetic acid to the benzidine–acetic acid reagent to increase its acid strength and claimed that it detected less than one microgram of glucose in free or combined form. Its sensitivity to fructose was considerably less. An acid is required for the formation of furfuraldehyde from sugars. Furfuraldehyde, or a degradation product of it, then reacts with an aromatic amine or phenol (benzidine in this case) to form a coloured product. It is important that the acid should be strong enough to cause the reaction to proceed, but, in the case of paper chromatography, it should not cause partial destruction of the paper or produce a dark background colour. For this purpose, organic acids (acetic, phthalic, or trichloracetic acid) have been used. However, when mineral acids at low concentrations are used, an improved reagent is obtained without paper destruction or serious background interference.

In an attempt to improve this reagent, it was discovered that addition of mineral acids to the benzidine–trichloracetic–acetic acid reagent caused precipitation, but this difficulty was overcome by the application of the components separately to the paper. The use of mineral acids with the benzidine reagent permits the detection of aldoses and ketoses in free or combined form in 1-mgm. quantities. Through the manipulation of the procedures to be described below, classes of simple sugars and monomers of oligosaccharides can be distinguished on one chromatogram. Procedure *a* is relatively specific for aldoses, and procedure *b* for ketoses. If both aldoses and ketoses are to be revealed in one chromatogram, procedure *c* or *d* should be used.

The benzidine reagent is prepared by dilution of 10 ml. of stock 1 (5·0 gm. benzidine in 100 ml. glacial acetic acid) plus 10 ml. of stock 2 (40·0 gm. trichloracetic acid in 100 ml. distilled water) to 100 ml. with 95 per cent ethanol[1]. The stock solutions keep indefinitely in brown bottles. The mineral acids are 0·3 *N* hydrochloric acid in 95 per cent ethanol and 1 *N* phosphoric acid in 95 per cent ethanol.

Procedure *a*: Spray a chromatogram with the benzidine reagent and heat at 70° C. for 10–15 min. Aldoses of 1 mgm. quantity in free or combined form are revealed as brown spots against a white background. Ketoses are detected only at high concentration.

Procedure *b*: Spray a chromatogram with the benzidine reagent. Follow this with a light spraying of 0·3 *N* hydrochloric acid in 95 per cent ethanol, and then heat at 70° C. for 10–15 min. Ketoses of 1 mgm. quantity in free or combined form are revealed as yellow spots against a pale background. Aldoses at high concentration give faint brown spots. The yellow colour intensity is enhanced under ultra-violet light.

Procedure *c*: It is essentially the same as procedure *b*, except that hydrochloric acid is replaced by 1 *N* phosphoric acid in 95 per cent ethanol. Both aldoses and ketoses in free or combined form are revealed in their characteristic colours on a chromatogram which appears moist and has a light background.

Procedure *d*: Spray a chromatogram with the benzidine reagent and heat at 70° C. for 10–15 min. for the detection of aldoses; spray lightly with 0·3 *N* hydrochloric acid in 95 per cent ethanol and heat again at 70° C. for 10–15 min. to detect ketoses. This procedure is better than procedure *c* because of its high sensitivity and relative specificity for classes of sugars. By this procedure both aldoses and ketoses in

either free or combined form are revealed as brown and yellow spots respectively against a light background. Stachyose, raffinose, cellobiose, melibiose, sucrose, maltose, lactose, rhamnose, galactose, glucose, fructose, mannose, arabinose, xylose and ribose give their characteristic colours, brown for aldoses, yellow for ketoses, and the colour of the oligosaccharides containing aldoses and ketoses depends on the sugar concentration and heating temperature.

By the addition of mineral acids at low concentration the benzidine reagent[1,2] is improved to detect simple sugars and oligosaccharides with high sensitivity and relative specificity.

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Crystallization of the Complex of D-Amino-Acid Oxidase and 'Substrate-Substitute'

In an earlier communication[1], it was reported that a crystal was obtained from the mixture of D-amino-acid oxidase apo-protein, flavin-adenine-dinucleotide and benzoate, a 'substrate-substitute'. It was also found that the crystal is composed of equimoles of the apo-protein, coenzyme and benzoate. By adding D-alanine to the solution of the crystal, the complete decolorization of the coenzyme accompanied by the liberation of the benzoate was observed. This result indicated that the benzoate can be replaced by the substrate, and that the enzyme is not denatured when crystallized by combining with benzoate. In the present experiment, we sought to find another 'substrate-substitute' to crystallize this enzyme.

Among the benzoic acid derivatives, *p*-nitrobenzoic acid was also considered to be a 'substrate-substituto' from our former experiment[2]. *p*-Nitrobenzoic acid was expected to be detected more easily by its absorption peak at 275 mμ than benzoate. Using *p*-nitrobenzoic acid as 'substrate-sui stitute', a crystal was obtained from the mixture of the apo-protein, flavin-adenine-dinucleotide and 'substrate-substitute' in the same way as reported in the previous report[1]. The solution of the crystal retains its full activity after heating at 60° C. for 3 min., indicating that the apo-protein is considerably stabilized in the crystal. The shape of the crystal is nearly the same as that of the crystal obtained from the mixture of the apo-protein, coenzyme and benzoate. The crystals were collected by filtration, and the filter cake was washed twice by distilled water. The crystals thus obtained were dissolved in pyrophosphate buffer (*M*/60, *p*H 8·3).

The absorption spectrum of the crystal in the visual wave-length region is shown by curve I in Fig. 1. It exhibits the same characteristic feature as in the case of the crystal of the apo-protein-coenzyme-benzoate complex: the peaks appeared at 380 mμ and 465 mμ, a marked shoulder being recorded at 485 mμ.

To liberate the combined coenzyme and the 'substrate-substitute' from the apo-protein, the *p*H of the solution of the crystal was adjusted to *p*H 5·0 and heated at 90° C. for 3 min. The completely denatured protein was removed by centrifugation. After the *p*H of the supernatant was corrected to 8·3, its absorption spectrum was measured as shown by curve II in Fig. 1. The spectrum was considered to be the sum of the spectra of two compounds, the coenzyme and *p*-nitrobenzoic acid. The spectrum, however, is identical with that of flavin-adenine-dinucleotide in the range from 370 mμ to the longer wave-length, and *p*-nitrobenzoic acid has no absorption in this region. So the theoretical spectrum of the coenzyme (curve III) was calculated from the optical density at 450 mμ of curve II. Consequently, the absorption spectrum of *p*-nitrobenzoic acid in the supernatant can be calculated to be curve IV, which has the same shape with that of the standard *p*-nitrobenzoic acid (curve V). Comparing the optical density at 275 mμ of curve IV with that of curve V, the molar concentration of *p*-nitrobenzoic acid as compared with those of the apo-protein and coenzyme in the crystal was calculated as follows: 1·2 moles of *p*-nitrobenzoic acid, 1·1 moles of the apo-protein, and 1·0 mole of flavin-adenine-dinucleotide.

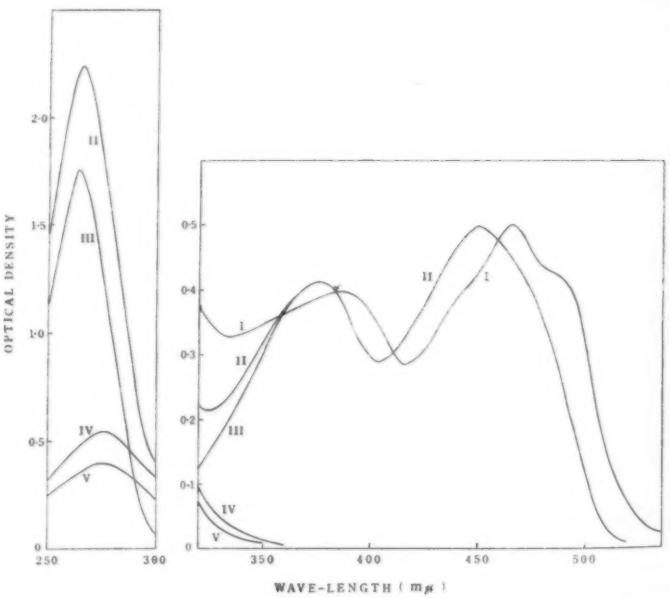

Fig. 1. Absorption spectra of the crystal and *p*-nitrobenzoic acid in pyrophosphate buffer (*M*/60, *p*H 8·3). I, The crystal; II, the supernatant obtained after heating I to denature the apo-protein (see text); III, flavin-adenine-dinucleotide calculated from the optical density of II at 450 mμ; IV, theoretical spectrum of II–III; V, *p*-nitrobenzoic acid ($4·0 \times 10^{-5}$ *M*)

From these results, it was concluded that D-amino acid oxidase apo-protein can also be crystallized by combining with equimoles of *p*-nitrobenzoic acid and coenzyme in the same way as in the case of benzoate. This fact may be attributed to the low specificity of this enzyme.

Furthermore, it seems clear that the enzyme can be easily crystallized by combining with a suitable compound as 'substrate-substitute' which can combine with the protein at the same binding sites as the substrate, but not be oxidized by the enzyme. The crystal of the complex of the enzyme and such a 'substrate-substitute' may provide a valuable clue in elucidating the actual features of enzyme-substrate compounds.

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PHYSIOLOGY

Persistence of Sterols other than Cholesterol in Chicken Tissues

RECENT work by Davison and his collaborators[1–3] has shown that cholesterol may be incorporated in the nervous tissues of myelinating chickens and rabbits and that this cholesterol will persist in these tissues for considerable lengths of time. Our interests in the metabolism of cholesterol and other sterols prompted us to compare the distributions in chicken tissues of injected sitosterol and lanosterol with that of cholesterol. Emulsions of cholesterol-7-^{3}H (purchased from New England Nuclear Co., Boston, Mass.), sitosterol-^{3}H (randomly labelled, gift of Dr. Leon Swell), and lanosterol-^{3}H (randomly labelled, gift of Dr. George J. Alexander) in saline were prepared by the method used for preparation of cholesterol-^{14}C emulsions for experiments involving mitochondrial oxidation of this sterol[4]. Newly hatched White Leghorn chicks were used and the appropriate sterol emulsion (0·50 ml.) was injected directly into the yolk sac. After four days, radioactivity could be detected in the brain tissue of chickens from each group. After 9 weeks the one surviving chicken in each group was killed by exsanguination. The blood was collected in centrifuge tubes and the erythrocytes separated. All the brain, cord, liver, spleen and aorta was excised, weighed and dissolved in strong potassium hydroxide. The non-saponifiable fraction was assayed for tritium radioactivity in a liquid scintillation counter. Small pieces of sciatic nerve were taken from each chicken and all showed slight but consistently detectable radioactivity. Other findings are shown in Table 1.

It is of interest that all three sterols were incorporated into brain tissue. The rather large incorporation into erythrocytes is also noteworthy as is the small but persistent incorporation of radioactivity into the aortic tissue. That a small percentage of orally ingested cholesterol will be deposited in the aorta of adult rabbit[5] or man[6] has been shown. The relative persistence of lanosterol-^{3}H in the liver could indicate greatly reduced turnover as compared with the other two sterols. In view of reports that some insects and mammals may convert sitosterol and ergosterol to cholesterol or cholesterol-like compounds[7–10], it was necessary to prove the presence of the injected material. Gas chromatography of the non-saponifiable fractions from the brains and livers of all three chickens showed the presence of only the injected sterol and cholesterol. (We are greatly indebted to Dr. W. M. Holmes of Smith, Kline and French Laboratories, Philadelphia, for these analyses.)

Table 1. RECOVERIES OF TRITIUM RADIOACTIVITY FROM NON-SAPONIFIABLE FRACTIONS OF CHICK TISSUES NINE WEEKS AFTER INJECTION OF CERTAIN STEROLS

Sterol	Recovery (per cent)		
	Cholesterol-^{3}H	Sitosterol-^{3}H	Lanosterol-^{3}H
Input (c.p.m.)	4·3 × 10^5	8·2 × 10^4	8·7 × 10^5
Brain	0·60	0·76	0·28
Cord	Lost	N.D.*	0·02
Erythrocytes	0·33	0·24	0·49
Liver	0·40	0·25	1·07
Spleen	0·14	N.D.*	0·35
Aorta	0·02	0·18	0·13

* N.D., not detectable.

Work with larger groups of chickens is in progress. Preliminary results are consistent with those presented here. Experiments involving more accurate characterization of the labelled sterols as well as autoradiography of specific tissues are under way.

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Effect of Sheep Pituitary Extract on Hypercholesterolæmia in Intact and Hypophysectomized Rats

THE prolonged administration of cortisone or adrenocorticotrophic hormone (ACTH) has sometimes been found to cause a change in the concentration of cholesterol in serum in human beings and in experimental animals. While some workers[1,4] report that they have found such an elevation, others have noted no effect[7]; still others have reported the opposite[3].

A study of endocrine factors influencing the cholesterol[8] content of serum has shown that, although a mild increase in the concentration of serum cholesterol occurred in rats from daily subcutaneous injection of 2·5 mgm. of cortisone acetate for two weeks, there was no significant change from corticotropin[5] in a dose of 0·3 mgm. (about 20 I.U.) suspended in peanut oil and given subcutaneously over the same period of time. However, in hypophysectomized rats there was an increase of 80 per cent or more in the cholesterol content of serum on treatment with 0·5 mgm. of cortisone acetate daily for a period of 4 days or with daily injection of 0·3 mgm. corticotropin in oil for two weeks. The effect of corticotropin was mediated through the adrenals as there was no such elevation in adrenalectomized hypophysectomized animals.

The increased sensitivity of the hypophysectomized rat, over the normal animal, to the development of hypercholesterolæmia from cortisone or corticotropin was found to be much greater in the case of hypercholesterolæmia induced by feeding 5 per cent cholesterol and 0·1 per cent sodium cholate in 'Purina' fox chow diet for periods of from four days to three weeks. A crude pituitary extract was found to lower the concentration of cholesterol under these circumstances.

Acetone-dried sheep pituitary powder was extracted by stirring with distilled water and adjusting the *p*H to 10 with 2·5 *N* sodium hydroxide; after centrifugation the extract was lyophilized. The dry material easily dissolved in saline at a concentration of 100 mgm. per ml. The animals were injected subcutaneously with a daily dose of 40 mgm. over the period of cortisone treatment, or over the last four days of feeding a high-cholesterol diet. Forty mgm. of this extract was given to hypophysectomized rats twice daily for four days. On the fifth day body weight and the weight of thyroid, adrenals, testes, seminal vesicles and ventral prostate were noted. It was found that the extract was rich in gonadotropins and, in addition, contained small amounts of growth hormone, thyrotropin and corticotropin.

None of the purified or semi-purified fractions of pituitary gland tested inhibited cortisone-induced or dietary hypercholesterolæmia completely. The fractions tested were growth hormone of Raben *et al.*[5,6] in a dose of 0·3–3·0 mgm. daily; an extract prepared by the method of Koenig and King[2] in a daily dose of 2–6 mgm.; Armour thyrotropin ('Thytropar'), 0·2 unit daily; Armour lactogenic hormone ('Panlitar'), 2–4 mgm. daily; Schering pregnant mare serum gonadotropin ('Anteron'), 20 I.U. daily; desiccated thyroid, 0·006–0·01 per cent in diet; and testosterone propionate, 2·5 mgm. twice weekly. Œstrogen increased somewhat the serum cholesterol in the rat. Animals given posterior pituitary powder in a dose of 5 mgm. daily all died by the third day. The extract of Koenig and King and desiccated thyroid had only a moderate effect alone or in combination. However, the effect of these compounds was more pronounced when given in higher doses. There was also a moderate effect noticed with testosterone and when bovine liver extract, prepared in a similar way and administered in the same dosage as the pituitary extract, was given (Table 1).

Table 1. Effect of Different Hormones on the Serum Cholesterol Content of Intact and Hypophysectomized Rats. For details of dosage and period of treatment, see text

	Normal (mgm. per cent)	Hypophysectomized (mgm. per cent)
Control	85 (117)*	73 (193)
ACTH	89 (8)	134 (10)
Growth hormone	84 (7)	73 (7)
ACTH + growth hormone	79 (7)	118 (6)
Cortisone alone	91 (5)	120 (18)
Cortisone + pituitary extract	—	71 (15)
Cortisone + growth hormone	—	181 (5)†
Cortisone + thyroxine (20 μgm. daily)	109 (6)†	150 (6)†
Cortisone + thyrotropin	—	127 (5)
Cortisone + Koenig's extract	—	113 (5)
Cortisone + chorionic gonadotropin	—	126 (5)
Cholesterol diet‡	285 (4)	373 (4)
Cholesterol diet + pituitary extract	121 (6)	124 (5)
Cholesterol diet + Koenig's extract	224 (7)	241 (5)
Cholesterol diet + testosterone	—	205 (6)
Cholesterol diet + desiccated thyroid (0·006 per cent)	—	306 (10)
Cholesterol diet + desiccated thyroid (0·01 per cent)	164 (10)	150 (7)
Cholesterol diet + liver extract	245 (5)	210 (5)

* Number of rats used.
† 2·5 mgm. of cortisone given daily.
‡ 5 per cent cholesterol diet for three weeks.

The doses used these fractions contained greater activity, as judged by the weight of the respective end organs in hypophysectomized rats, than was obtained from alkaline extract of sheep pituitary powder.

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Antagonism of Reserpine by Intraventricular Bretylium

Bretylium, as shown by Boura and Green[1], causes an inhibition of sympathetic nervous transmission by a blocking action at adrenergic postganglionic neurons. Reserpine also inhibits peripheral sympathetic nervous transmission but does so by depleting adrenergic nerve endings of the transmitter substance norepinephrine[2]. It also causes a depression of central nervous activity (tranquillization or sedation) which is associated with depletion of brain stores of norepinephrine[3] and 5-hydroxytryptamine[4]. It seemed of interest to see if bretylium could block the actions of reserpine on the central nervous system. In order to study interactions of the two drugs on the central nervous system both had to be given so that effective concentrations of each could be attained at this site. Investigations by Boura *et al.*[5] in which labelled bretylium, which is a quaternary amine, was administered to cats, demonstrated that only small quantities reached the central nervous system. In order to attain a higher concentration the bretylium in the present work was administered intraventricularly.

Male albino mice of *CF*–1 strain were used. Bretylium tosylate, 4 mgm./kgm. dissolved in normal saline, was given subcutaneously or in a single intraventricular injection of 0·03–0·05 ml. following the method described by Haley and McCormick[6]. Control intraventricular injections of equal volumes of normal saline were also made. Reserpine phosphate, 2·5 mgm./kgm., was injected subcutaneously 0·5 hr. after the intraventricular injection. The mice were kept in individual jars. The following tests were begun on the mice 0·5 hr. after the reserpine injection: (1) *spontaneous activity:* as each jar cover was removed the mouse was scored 100 (moving body), 50 (moving head), or 0 (no movement); (2) *evoked*

Table 1. Effect of Intraventricular Bretylium on Reserpine in Mice

Treatment No. 1 (intraventricular)	Treatment No. 2 (subcutaneous)	No. of mice	Time tested after treatment No. 2: ½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
			Blepharospasm (complete closure = 100)				
Bretylium	—	5	0	20	0	0	0
Saline	—	17	0	0	0	0	0
—	Reserpine	15	0	6	30	52	67
Bretylium	Reserpine	15	0	7	12	13	23
Saline	Reserpine	20	0	6	30	44	56
—	Reserpine plus bretylium	5	0	0	0	53	80
			Plasticity (complete immobility = 100)				
Bretylium	—	5	100	90	90	20	0
Saline	—	17	0	47	29	24	8
—	Reserpine	15	0	30	30	73	90
Bretylium	Reserpine	15	75	90	60	47	60
Saline	Reserpine	20	95	63	70	68	80
—	Reserpine plus bretylium	5	40	20	30	80	100
			Evoked activity (normal mouse = 100)				
Bretylium	—	5	60	80	100	100	100
Saline	—	17	86	88	91	94	92
—	Reserpine	15	90	90	63	37	17
Bretylium	Reserpine	15	40	40	47	57	53
Saline	Reserpine	20	87	83	40	33	25
—	Reserpine plus bretylium	5	90	100	90	50	0
			Spontaneous activity (normal mouse during first ½ hr. = 100)				
Bretylium	—	5	50	0	20	60	80
Saline	—	17	93	41	29	32	38
—	Reserpine	15	95	57	50	30	10
Bretylium	Reserpine	15	0	0	23	33	30
Saline	Reserpine	20	17	10	5	10	0
—	Reserpine plus bretylium	5	100	80	60	70	40

activity: the mouse was picked up by the tail and scored 100 (marked sideways movement or arching of body), 50 (slight movements of body), or 0 (no movement); (3) *blepharospasm:* while held by the tail the degree of opening of the palpebral fissure was scored 100 (complete closure), 67 (almost closed), 33 (slightly narrowed), or 0 (wide open); (4) *plasticity:* the mouse was replaced in the jar in a semi-standing position with its forefeet resting on the vertical sides of the jar and the degree of plasticity or immobility in this unusual position was scored 100 (retains unusual position for more than 15 sec.), 50 (retains unusual position for 5–15 sec.), or 0 (gets back on all four feet in less than 5 sec.). The scores resulting from each series of mice are given in Table 1.

The most marked effect observed was the block by intraventricular bretylium of the reserpine induced blepharospasm. Four hours after reserpine all mice without intraventricular bretylium had marked or complete blepharospasm (scores 67 or 100) while those with bretylium ranged from no closure to marked blepharospasm. Subcutaneous bretylium was not effective in counteracting this action of reserpine.

The intraventricular injections of saline or bretylium produced some plasticity, as defined above, in the responses of the mice. This was more marked with bretylium, but disappeared over the 4-hr. test period. Reserpine had a slow onset, gradually producing plasticity, and this appeared to be inhibited by intraventricular bretylium.

At 4-hr., evoked activity of reserpine-treated mice was depressed. Subcutaneous bretylium and reserpine also blocked evoked activity. Intraventricular bretylium did not depress evoked activity of the mice and tended to counteract the depression caused by reserpine.

Spontaneous activity was the most variable parameter tested. Mice receiving reserpine without bretylium showed somewhat less activity at 4 hr. than those receiving intraventricular bretylium in addition to reserpine.

The interpretation which we would give to the above results is that bretylium is capable of antagonizing the central effects of reserpine if it is injected so that it reaches the same sites of action as reserpine. This correlates with the interaction of bretylium and reserpine peripherally reported recently by Costa[7], who has shown that when the two drugs are administered together the ability of reserpine to deplete cardiac stores of norepinephrine is markedly reduced.

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Effect of Aldosterone on the Living Cornea *in vitro*

Earlier work has shown that the cornea of the rabbit, excised and mounted between two chambers, can be kept alive and functioning for 8 hr.[1] during which time it can be demonstrated that the epithelium generates an electrical potential of 20–50 mV.[1], that sodium is actively transported across the epithelium into the corneal stroma[2], and that the net flux of sodium is equivalent to virtually all the neutralizing current[2,3]. This system appeared to afford a satisfactory model for the *in vitro* study of the effect on sodium transport of aldosterone, the principal sodium-retaining steroid of the adrenal cortex of most mammals studied[4]. Aldosterone is assumed to affect sodium exchange in the renal tubule[5], and has been demonstrated *in vitro* to stimulate transport of sodium across the isolated bladder of a toad[6] and skin of a frog[7]. In the present investigation the effect of aldosterone on the excised cornea of the rabbit was assessed *in vitro*.

The cornea was excised and mounted in a lucite clamp between two chambers, each containing a facsimile of rabbit aqueous humour[1]. (The composition of this fluid was the same as that used in the earlier

work[1,2] except that 2·0 instead of 1·0 ml. of glucose 12 per cent were added. Ascorbic acid (20 mgm. per cent) was added in several experiments, also[3].) The pressure within the chamber was adjusted to approximate normal intraocular pressure, 20 cm. of water. Thirty experiments were carried out with measurements of potential difference, electrical resistance, and neutralizing current being made at 20-min. intervals. The preparation was allowed to stabilize over a 2-hr. period. Measurements were then made over an additional hour to establish a base-line for readings during the experimental period. At the end of the hour, *d*-aldosterone (kindly provided by Dr. Robert Gaunt of CIBA, Inc.) was added to the bathing medium, in 15 instances to the epithelial side, and in 15 to the endothelial side. The *d*-aldosterone was dissolved in distilled water. The steroid was added to the preparation in such quantities as to arrive at a final concentration in the ambient fluid of 1–1,000 μgm./100 ml., that is, in concentrations ranging from 10^2 to 10^5 times those encountered under physiological conditions. Such high concentrations are effective in enhancing sodium balance *in vivo*[4] and sodium transport *in vitro*[7]. In two experiments, cortisol, dissolved in small quantities (0·1–1·0 per cent, final concentration) of ethanol, was added in addition to aldosterone, in order to assay a possible 'permissive' effect of cortisol on aldosterone action, as shown in the whole organism[5].

In order to provide for optimal diffusion and tissue binding of the steroid, 2–3 hr. were allowed to pass. In two instances, tritiated aldosterone was added to the bathing fluid in order to determine the permeability of the cornea to steroid. At the end of a 5-hr. period, fluid was removed from both sides of the cornea, carrier aldosterone was added, the fluid specimens were extracted with ethyl acetate, and the extracts were washed, dried, and chromatographed in the Bush *B5* system (benzene : methanol : water)[10], the steroids being localized by reaction with blue tetrazolium and eluates counted in a liquid scintillation counter.

Table 1. Percentage Change in Electrical Indices of Rabbit Cornea *in vitro* during the Addition of *d*-Aldosterone*

Exp. No.	Experimental conditions: Compound added to fluid bathing epithelium	Electrical indices: % Δ *I*	% Δ *PD*	% Δ *R*
		(per cent)		
1	20 μgm. aldosterone	0	+ 4	+ 9
2	1 μgm. aldosterone	− 2	+10	+14
3	10 μgm. aldosterone	− 4	− 6	− 9
4	20 μgm. aldosterone	+28	+58	+18
5	10 μgm. aldosterone	+ 3	+ 8	+ 8
6	10 μgm. aldosterone	+ 8	+10	+ 1
	Compounds added to fluid bathing endothelium			
		(per cent)		
7	10 μgm. aldosterone 20 mgm. sodium ascorbate	+ 2	+ 9	+ 6
8	10 μgm. aldosterone	− 5	+ 6	+ 5
9	10 μgm. aldosterone 20 mgm. sodium ascorbate	−14	+ 7	+26
10	10 μgm. aldosterone 1 mgm. cortisol	− 4	+ 7	+10
11	10 μgm. aldosterone 1 mgm. cortisol	+ 1	− 4	− 5
12	100 μgm. aldosterone 10 mgm. cortisol	+11	+14	+20
13	Control—nothing added	−14	+ 6	+21

* These figures represent percentage changes in current (*I*), potential difference (*PD*) and resistance (*R*) from a time one half-hour before the addition of aldosterone to a time 2 hr. after addition of the hormone. There is no large or consistent change in neutralizing current (sodium flux). Experience has shown that the small increases in *PD* and *R* cannot be interpreted as indicating anything more than the viability of the preparations.

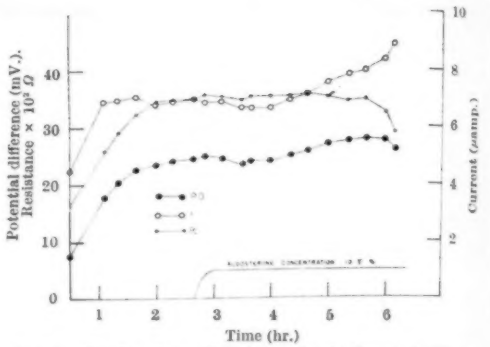

Fig. 1. Results of experiment 6. No significant changes in electrical indices can be found in response to the addition of aldosterone to the bathing media. The changes at the far right of the graph indicate the end of the useful life of the preparations

The results of 13 representative experiments are summarized in Table 1, and one of these experiments (No. 6) is illustrated in Fig. 1. It will be noted that the addition of *d*-aldosterone over a wide range of concentrations elicited no significant change in any parameter of electrical activity, that is, addition of the steroid did not enhance the active transport of sodium. Further, addition of cortisol was without effect. Aldosterone was shown to pass through the cornea, the concentration on the opposite side being about 1/100 that on the side to which the steroid was originally added.

The results do not agree with those of Crabbé[6] or of Penney *et al.*[7]. They are, however, in harmony with those of Maetz *et al.*[11], who failed to demonstrate an enhancement by aldosterone of active sodium transport in the skin of a frog *in vitro*, but showed such an enhancement in the isolated skin of frogs which had been pre-treated with the steroid. A possible explanation for the lack of *in vitro* effect of aldosterone in this preparation is that more than 6 hr. may be required for such an effect to become manifest. However, it is not possible to keep the rabbit cornea alive and functioning effectively much in excess of 6 hr. An alternative hypothesis is that sodium transport in this multi-layered, avascular membrane occurs in large part independently of hormonal control. There is no *a priori* reason for supposing that aldosterone acts ubiquitously on cellular membranes.

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HÆMATOLOGY

Use of Phenol in the Isolation of Erythropoietic Glycoprotein

THE erythropoietic activity in the plasma of rabbits made anæmic with phenylhydrazine can be isolated as an acid glycoprotein. A dialysed and lyophilized ethanol fraction from heat-coagulated plasma consistently gives us a potent stable highly water-soluble preparation. Six laboratories have found good correlation in a comparative assay of a batch prepared by us[1]. Further di-ethyl-amino-ethyl cellulose column fractionation removes 40–50 per cent of inert protein and results in the active glycoprotein previously described[2].

While the active peak could be re-chromatographed on di-ethyl-amino-ethyl cellulose without apparent change in electrophoretic and elution properties, there was usually a drop of 25–75 per cent in specific activity. Even more striking losses occurred in the fractionation of erythropoietin from human urines. Urine from patients with congenital hypoplastic anæmia was precipitated with 4 volumes of ethanol. The non-dialysable portion of the precipitate, a mixture consisting in the main of glycoproteins, contained essentially all the erythropoietic activity of the original urine. It could be further resolved on di-ethyl-amino-ethyl cellulose but with variable, sometimes complete, loss of activity.

One ethanol precipitate from human urine, after removal of the dialysable part, had this activity: in a fasted rat assay[1] 1 mgm. total dose per rat gave 26·8 per cent ^{59}Fe-uptake in the blood. When 100 mgm. portions of the precipitate were fractionated on di-ethyl-amino-ethyl cellulose we repeatedly lost 90–100 per cent of the activity although all the protein was recovered in successive eluates with 0·01 to 1·0 *M* sodium chloride.

We had observed before that plasma erythropoietin is not damaged by phenol and is extractable into a phenol upper layer like some other glycoproteins in the procedures of Morgan and Partridge[3], Westphal *et al.*[4] and Kirby[5]. By using as little as 0·1 per cent phenol in all equilibration and elution buffers in di-ethyl-amino-ethyl cellulose chromatography, as well as on both sides of the membrane during dialysis, we are now consistently recovering more than 50 per cent of the activity contained in the original urine.

The dialysed and lyophilized ethanol fraction from plasma, fractionated as previously described on di-ethyl-amino-ethyl cellulose[2], but in the presence of phenol, yields a peak with 2–3 times the specific activity of the original fraction, corresponding with the removal of inert material.

Evidence to date indicates that erythropoietic activity requires integrity of a glycoprotein since proteolytic enzymes as well as neuraminidase inactivate. Erythropoietin could be a glycoprotein associated with inert glycoprotein or even some other compound which becomes labile on disintegration of carrier glycoprotein. Enzymes like those mentioned, or other as yet undefined carbohydrases, either secreted with the urine or coming from other sources, could be responsible for large losses of urinary activity. The presence of phenol during isolation may prevent subtle changes through bacteriostasis, enzyme inactivation, anti-oxidant action, etc.

If it were not for measurement of the biological activity the protective effect of phenol would not have been noted. We hope that this preliminary report might be useful to those working with glycoproteins with or without biological activities.

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Thalassæmia in Melanesia

THALASSÆMIA has previously been regarded as non-existent in Melanesians[1]. In August 1960, a Papuan female from the Milne Bay area of New Guinea, aged 18–20 yr., was admitted to the General Hospital, Port Moresby, for re-investigation of a refractory anæmia first noticed during pregnancy in 1957. On that occasion she had been delivered of a full-term, apparently normal, infant, her hæmoglobin-level having been maintained by transfusions. Since discharge she had received no treatment and had been able to carry on normal village life, in spite of a hæmoglobin-level remaining fairly constant at 5–6 gm. per cent. Investigation in August 1960 showed typical bony changes, a hypochromic microcytic anæmia with target cells and normoblasts present in the peripheral blood, and the alkali denaturation test[2] showed 67 per cent undenatured hæmoglobin. The presence of hæmoglobin *F* as a major component was confirmed by hæmoglobin electrophoresis on cellulose acetate strips. Since then three other cases of thalassæmia major in Papuan children have been diagnosed, two of the children aged 5 yr. and 1·5 yr. came from the Kerema district, and one aged 3 yr. from the Oro Bay area in Papua.

All showed typical bony changes on radiological examination, a hypochromic microcytic anæmia with normoblasts and target cells in the peripheral blood and excess hæmoglobin *F*—32 per cent, 39 per cent and 46 per cent—by alkali denaturation, confirmed by hæmoglobin electrophoresis. All had been treated by transfusion on many occasions. In two of them diagnosis was delayed, presumably in one because of suppression of endogenous erythropoiesis by transfusion[3], and in the other by the presence of an aplastic crisis[4]. One of the children from Kerema has undergone splenectomy and his transfusion requirements have been greatly diminished since operation. Glucose-6-phosphate dehydrogenase estimation in this child on two occasions showed 46 and 56 units/100 ml. red blood cells respectively, suggesting that he has the negroid type of glucose-6-phosphate dehydrogenase deficiency in addition to thalassæmia.

In view of the fact that these cases have come from widely separated districts, it is considered that thalassæmia may be widespread in New Guinea.

Thanks are due to Dr. P. Brain of the Red Cross Transfusion Service, Perth, who carried out the hæmoglobin electrophoreses, Dr. D. Curnow and

Dr. W. Pitney, of the Royal Perth Hospital, Perth, who performed the alkali denaturation tests and examined the peripheral blood and marrow smears, and Prof. J. B. Chatterjea of the School of Tropical Medicine, Calcutta, who confirmed the presence of hæmoglobin *F* in two of the cases, being the first to demonstrate it in one case. Mr. Ian Parsons, of the Peter MacCallum Institute, Melbourne, carried out the estimations of glucose-6-phosphate dehydrogenase.

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HISTOLOGY

Antidiuretic Hormone and Renal Collecting Tubules

Ginetzinsky[1] has reported that, in the kidneys of rats treated with posterior pituitary extract, the epithelium of the collecting tubules becomes flattened and its basement membrane less distinct. We have attempted to repeat Ginetzinsky's observations.

Fifty-two young male white rats, 150–200 gm. in weight, were fasted overnight with free access to water and then treated in one of four ways:

(1) Eighteen rats were given water by stomach tube (5 per cent of body-weight followed by 3 per cent after 1 hr., or 7 per cent of body-weight with replacement of lost water every ½ hr.) and were killed during a diuresis of 2·0–3·5 ml. (11 rats) or 1·0–2·0 ml. (7 rats) per ½ hr.

(2) Sixteen rats were treated similarly, but the water diuresis was inhibited by a subcutaneous injection of 'Pitressin' (Parke Davis), 25–30 m.units per 100 gm. body-weight. These rats were killed when urine flow, which was rapidly inhibited, had fallen to 0·3 ml./15 min. or less; in 4 rats the flow had ceased completely for a half-hour or more.

(3) Nine rats were given the hormone but no water, and (4) nine were given neither water nor hormone but killed after being handled similarly to the other groups. In groups (3) and (4) the output of urine did not exceed 0·3 ml. in any single 15-min. period.

The following methods were used for staining sections of kidneys of these rats: (*a*) Bharadwaj and Love's method for mitochondria[2]; (*b*) LaCour, Chayen and Gahan's method for lipids[3]; fixation in Zenker's fluid, followed by (*c*) hæmatoxylin and eosin, (*d*) Heidenhain's azan, (*e*) toluidine blue or (*f*) the periodic acid–Schiff reaction (PAS); (*g*) fixation in formol saline, followed by Heidenhain's iron hæmatoxylin. In each group, Bharadwaj and Love's method, the PAS reaction and either azan or hæmatoxylin and eosin were each used for at least half the rats and the other methods for two animals of the group.

The staining of mitochondria and lipids showed differences between rats and between tubules in individual kidneys. These variations are reported elsewhere[9].

From the examination of sections stained by the PAS reaction, by toluidine blue, by azan, and by Heidenhain's iron hæmatoxylin, the following generalizations can be made:

(1) In all rats the collecting ducts were lined by cuboidal epithelium, which became low columnar in form near the tip of the pyramid. The height of the epithelium of these ducts did not differ perceptibly from one rat to another or between groups of rats, if similar distances from the tips of the pyramids were taken in choosing fields for comparison.

(2) The collecting ducts converge early in their course, so that sections parallel with the plane of the pelvis and ureter must be central if the ducts are to be seen in the pelvic half of the pyramid. Besides these ducts, the only tubular organs in the innermost medulla are blood vessels and thin segments of Henle's loops. Thus it is possible to obtain longitudinal sections of the pelvic half of the medulla which show only tubules lined by low cells, such as those in Ginetzinsky's Fig. 4, but it does not follow that some of these must be collecting ducts, which in this region are to be found only in the centre of the pyramid.

(3) The basement membranes varied in appearance between sections of the same block when toluidine blue was used, but, after the PAS reaction, these membranes were clearly seen, constant in appearance for each part of the nephron, and did not vary appreciably between the experimental groups.

(4) The PAS reaction showed the collecting ducts to differ between individual kidneys in the proportion of intercellular boundaries which showed stained cement. In the ducts of some kidneys PAS-positive material was present in the lumina in masses up to 50μ in diameter. These two features were not obviously correlated with each other, nor did either provide a point of distinction between experimental groups.

These findings do not support the suggestion that hyaluronidase, which Ginetzinsky[1,6] reported to be secreted in increased concentration in the urine under the influence of antidiuretic hormone, acts by altering the visible form of basement membranes and intercellular cement in the collecting ducts. Since such variability in tubular mucopolysaccharides as was found in our experiments could not be correlated with the treatment of the rats, our results may be compared with those of Berlyne[4,5], who, estimating hyaluronidase in the presence of a constant concentration of electrolytes[7], reported that the rate of excretion of hyaluronidase in the urine was unrelated to the rate of urine flow. We did not obtain any evidence for the apocrine activity which, according to Ginetzinsky[1], is indicated by a loss in height of the epithelium of the collecting ducts.

The findings reported here agree with those of Heller and Lojda[8], who examined renal histology under the influence of water and antidiuretic hormone but did not record rates of urine flow.

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PATHOLOGY

Acetylcholine in Sweat in Fibrocystic Disease of the Pancreas

THE varied secretory abnormalities in fibrocystic disease of the pancreas in which elevated sweat electrolytes and deficient pancreatic enzymes are linked to viscid mucus glycoproteins and increased parotid secretory rate suggest an underlying mechanism of overstimulation of exocrine glands with cholinergic innervation[1-6]. Roberts[5] further hypothesizes that excessive exocrine gland stimulation might result should acetylcholine (ACH), released at secretory nerve endings, persist owing to defective enzymic breakdown by cholinesterase, or, alternatively, to excessive quantities released. It seemed to us that a stimulating neurohumor not completely inactivated at its site of release might be found in the secretion of the gland it stimulates. Thus we examined sweat, a secretion abnormal in fibrocystic disease, for evidence of any characteristic neurohumoral activity. We have found in thermal sweat well-defined levels of a free choline-ester indistinguishable from acetylcholine. The activity was detected in sweat samples from normal individuals as well, but appeared to be of a significantly higher order in the fibrocystic.

Patient subjects were twenty-nine children in hospital at the National Institutes of Health, sixteen with and thirteen without fibrocystic disease of the pancreas. Their average age was eight years. Each was sweated thermally for 1 hr. in a plastic bag under blankets by a modification of Schwachman's method[6], in which collections were made from a restricted area

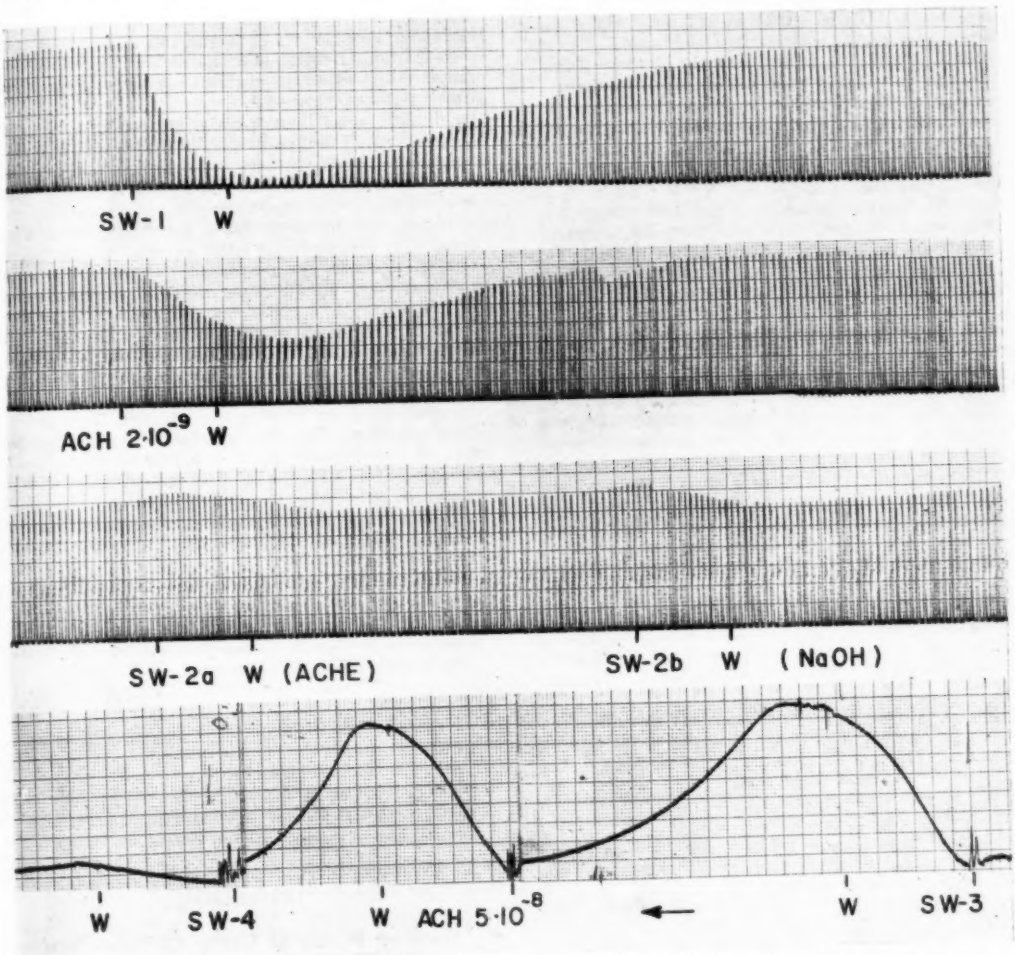

Fig. 1. Bioassay (*Venus* heart and frog rectus) of sweat from fibrocystic patient (lowest tracing reads right to left). *SW*-1, 0·4 ml. of sweat inhibiting *Venus* heart 95 per cent, giving estimated ACH content of 0·1 μgm./ml. of sample; *W*, wash, at 1·5 min. with *Venus* heart, 2 min. with frog rectus assay; ACH 2×10^{-9}, acetylcholine chloride, final concentration in bath of solution containing 0·02 μgm./ml. giving 50 per cent inhibition of heart; *SW*-2*a*–*SW*-2*b*, 0·4 ml. vol. of sweat inactivated by acetylcholinesterase (*SW*-2*a*) and sodium hydroxide (*SW*-2*b*); *SW*-3, 1·0 ml. of sweat giving 43 mm. contraction of frog rectus muscle, equivalent, after correction for interfering activity to ACH content of 0·15 μgm./ml. (several constituents of sweat affect the frog rectus contraction—we have found the highly specific *Venus*-heart assay gives a more accurate ACH level)—0·5 ml. of sweat gave a 17-mm. contraction, not shown); ACH 5×10^{-8}, acetylcholine chloride, final concentration in bath of solution containing 0·25 μgm./ml., giving 38 mm. contraction of muscle; *SW*-4, 0·55 ml. of sweat inactivated for ACH with acetylcholinesterase treatment; 4 mm. contraction from non-inactivated substances remains

of the back and simultaneously from the rest of the body surface. Sweat obtained by another method[7] and for shorter time-periods gave similar results. Measurement was made of sweat volume, *p*H, sodium and potassium. Samples were acidified to *p*H 4·0 with 1 *N* hydrochloric acid and assayed immediately or frozen to preserve activity. Sweat was tested in volumes of 1 ml. or less for choline-ester activity by bioassay on the heart of *Venus mercenaria* according to Welsh's method[8] and occasionally, when feasible, on the eserinized frog rectus muscle[9].

Two inactivation procedures used on each sample distinguished choline-ester activity from that of choline and inorganic ions. Activity was abolished by boiling for 15 min., at *p*H 12·0, after addition of sodium hydroxide but preserved after boiling at acid *p*H 4·0 (ref. 9). In the second procedure the activity was destroyed on incubation for 1 hr. with 0·5 mgm. of commercially purified bovine erythrocyte cholinesterase (ACHE) in buffer at *p*H 6·8. *p*H measurements were made with a glass electrode.

Fig. 1 shows an example of the activity found in sweat from a representative fibrocystic patient. The average choline-ester activity for the sixteen fibrocystic patients expressed as acetylcholine was 0·075 μgm./ml. of sweat (range 0·01–0·25 μgm./ml.). The corresponding figure for the thirteen control patients was 0·0065 μgm./ml. (range 0·002–0·02 μgm./ml.). Five fibrocystic patients gave very high values averaging 0·19 μgm./ml. Some scatter appears in the data from the disease group. Also overlap occurs between groups as one fibrocystic failed to give levels higher than 0·01 μgm./ml. while two non-fibrocystics presented levels of 0·015 and 0·020 μgm./ml. Levels tended to be constant on retesting the same individual and did not appear to be related to sweat volume or *p*H.

Our work has not been extended to adults or to infants, nor is it controlled for variable degrees of acclimatization or for the finer physiological parameters of sweat collection. The relationship of the findings to function of sweat glands therefore remains to be clarified. Two previous reports describe acetylcholine in sweat from normal individuals in levels estimated at 0·001–0·002 μgm./ml. (ref. 10). The presence of this neurohumor in remarkably higher amounts in the sweat of fibrocystic patients is being investigated.

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Types of Cell in Ascites Tumours

The mouse tumour *MCIM* (Klein) exists in two sub-lines, the M_{SS}, which only grows in the solid form, and the M_{AA}, which grows as an ascites tumour and also in solid form, designated M_{AS}. The difference between the behaviour of the sub-lines is as yet unexplained in terms of cell structure. Bergstrand and Ringertz have recently found no constant difference on examination with the electron microscope[1]. These tumours have been investigated recently by means of the aniline-blue staining technique[2].

It has been found that solid tissue cells, including those of solid tumours, show a blue rim- or surface-staining which is believed to be associated with staining of the common part of the cell walls of adjacent cells which lie between the electron-dense cell membranes. In the M_{SS} tumour such staining is seen in fresh preparations and in sections. The fresh ascites cells of the M_{AA} tumour, like all ascites tumour cells examined, fail to stain at all with the aniline-blue reagent, and stand out as clear, refractile spheres in the blue solution. However, there are also present in the fluid, and sometimes loosely adherent to the peritoneum, small compact masses of cells which, when washed to remove loosely attached free cells, are firm in consistency and show a blue staining surface which may be thick and irregular.

When fresh preparations of the M_{AS} tumour are so treated, it is found that both types of cell are present but in different proportions. There is, in young tumours, a preponderance of solid tumour masses which show blue-staining surfaces, with isolated pools of 'free' cells which can readily be washed out from the tissue and which fail to stain. It appears, therefore, that tumours of either type derived from the $_{AA}$ sub-line contain both elements, the predominating form depending on the environment. This accounts for the appearance of apparently typical 'ascites' cells in the M_{AS} tumours examined by Bergstrand and Ringertz. The softening found in the centre of older M_{SS} tumours is probably due to the cell degeneration so commonly found; in the M_{AS} tumours there may, in addition, be a predominance of 'free' cells in such areas.

Whether the two forms of cell breed true or whether the surface changes are reversible is not yet clear. Further investigations into this and into the reason for the failure of the free ascites cells to stain with aniline-blue are in progress.

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BIOLOGY

Feeding Habit of Some Central African Freshwater Fishes

Fishes of the family Citharinidae, belonging to the genera *Phago* Steind., *Eugnathichthys* Blgr. and *Belonophago* Gilt. (Figs. 1–2), together forming part of the sub-family Ichthyoborinae, have an extremely specialized and curious feeding-habit, being, namely, fin-eaters.

This most interesting fact was discovered during an ichthyological survey of Lake Tumba and the Central Congo Basin by our Institute in 1959. Amongst other biological observations, the examination of all gut contents was particularly stressed, and a large number of specimens belonging to these genera were found to have fin fragments in their stomachs. All species belonging to these genera apparently are exclusively fin-eating, at least in the adult stage. (Only *Phago loricatus* Günth., from the Niger, has not been examined.) Other genera, like *Gavialocharax* Pell. and *Paraphago* Blgr., judging from their morphology (dentition), probably also belong to this interesting little group, while *Phagoborus* Myers., though ichthyophagous, also has been found to eat fins occasionally.

Several authors, amongst others G. Marlier[3], had already noticed that most fishes in the Central Congo rivers had mutilated fins, often even severely torn and cut up. This condition was thought to be caused by some carnivorous species of leech, insect or fish, but was mostly ascribed to bacteria or some type of fin rot, and at first we put forward the hypothesis that it was done by a large predaceous Dytiscid beetle (*Cybister* sp.), which often attacks (small) fish. Mr. J. P. Gosse, of the I.N.E.A.C., had also recently noticed this strange fin-eating habit in some of these species of fish, thus further confirming our observations.

Morphologically, these fish are very specialized and possess a powerful dentition, well adapted to their method of feeding. They are very elongate species, *Belonophago* even needle-like in form. *Phago* and *Belonophago* are very slender, possessing large, extremely hard scales, often strongly ridged; their jaws are long, narrow and beak-like. *Eugnathichthys* has numerous small soft ctenoid scales and shorter, broad and strong jaws. The first two genera are small, respectively reaching a size of 16 and 12 cm., whereas *Eugnathichtys* can grow to 30 cm.

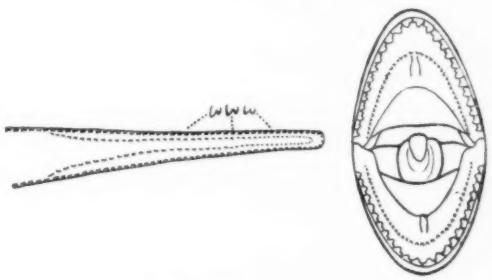
The mouth is large, the jaws elongate, closing tightly on one another; the premaxillary is mobile.

The teeth are cuspidate (bicuspid, usually), in two parallel rows, those in the outer one much larger, strong, sharp and firmly inserted on the external border of the jaw, forming a saw-like ridge. This last character is particularly apparent in *Eugnathichthys*, the dentition of which reminds one of that of South American Piranhas (*Serrasalmus*).

The intestine is very short, nearly straight, being coiled back only once on itself, with numerous pyloric cæca; the stomach is large and muscular. In spite of the bony nature of the material ingested, digestion seems to be fairly rapid, to judge from the fact that many specimens had empty stomachs. The stomach contents are composed exclusively of diverse fin fragments and some scales, the intestine containing only hard concretions, largely of a calcareous nature. The young (of less than 30–40 mm. standard length), feed mostly on aquatic insect larvæ (*Ephemerida, Trichoptera*).

Observations effected in a large aquarium containing a specimen of *Belonophago hutsebauti* Gilt. and several other fish species (*Distichodus, Chrysichthys* spp.) revealed some interesting aspects of its mode of feeding. The fish 'hovered' in horizontal position just below some floating grass, the fins (especially the pectorals) vibrating. As soon as some other fish swam within reach, it darted out, grabbed hold of a fin between its long jaws and snipped off part with a quick twist, the startled victim unconsciously favouring this action by also twisting and pulling away . . . minus a piece of fin.

Fig. 1. *Belonophago tinanti* Poll. (× 2/9) (ref. 10)



(*a*) (*b*)

Fig. 2. (*a*) *B. tinanti*; upper jaw, showing dentition (ref. 10). (*b*) *E. eetveldi*; mouth, showing dentition (ref. 10)

The species belonging to the genera mentioned often go about in small groups in lakes and large rivers, close along the shores, and preferably in relatively calm zones where vegetation (bushes, grass) trails in the water, or near the expanses of floating grass (*Echinochloa* and *Panicum*) which clog up the river arms and coves. The species attacked usually are slow-moving herbivores like *Distichodus, Tylochromis*, or bottom-feeders like *Chrysichthys*, though more vigorous swimmers like *Alestes, Lates* and even *Hydrocyon* often also show lacerated fins. The dorsal, anal and caudal fins are those commonly bitten off, paired fins rarely showing mutilations. Moreover, according to the native fishermen, they appear to attack man on some occasions.

A similar feeding habit has been suspected for the Nyassa cichlid fish *Docimodus johnstoni* Blgr., the strong jaws and cutting teeth of which are figured by Boulenger[2]. Of this it is written[1]: "This fish is well named Chiluma—'the thing that bites'. Stomach contents suggest that the cutting teeth may be used to bite pieces from the fins of other fishes". Trewavas states (personal communication) that of eleven stomachs examined six were empty, four contained fragments of fins and one a little plant and insect débris. Among marine fishes the Blennidae *Runula*[4] and *Aspidontus*[5] are described as parasites, biting bits off fins and skin of other fishes, *Aspidontus* approaching its victim by means of a mimetic resemblance to a 'cleaner-fish'. An allied habit is that of scale-eating, practised not only by certain Cichlidae of lakes Tanganyika[6,9] and Nyasa[2,6,11] but also by a Panamanian characid, *Roeboides occidentalis*[3].

Considered economically, these fishes are a nuisance in the sense that the wounds inflicted not only pave the way for various diseases (especially bacterial and fungal), like fin rot, but often contribute to make the victim an easy prey for predators, some having their fins nearly entirely amputated, thus considerably hampering their locomotion and balance.

Fin-eating fish are very common in some places, for example, in Lake Tumba, where many species show serious fin mutilations. Unfortunately, there exists no effective method actually of reducing their numbers, as they are hard to catch with conventional fishing gear. Small-meshed gill-nets, however, placed perpendicularly to the shore in appropriate zones, often catch quite a number, but have the disadvantage

of also catching the young of many economic species, at least in certain seasons.

This curious mode of feeding almost certainly evolved from the voracious habits of their ancestors, this being supported by the fact that related genera, like *Mesoborus* Pell. and *Phagoborus*, for example, are distinctly ichthyophagous, the latter even partly fin-eating occasionally, whereas all members of the sub-family Ichthyoborinae are predominantly carnivorous.

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Mediterranean Fishes of the Family Istiophoridae

Scombroid fishes belonging to the family Istiophoridae are rather uncommon in the Mediterranean. However, a species has been known for a long time and was actually discovered there. It is *Tetrapturus belone*, described by Rafinesque[1] from Sicily, where it is captured chiefly during the summer months; later, it was found in other localities, for example, Spain, Genoa, Naples, Malta and the Adriatic.

A second Istiophorid was described by Canestrini[2] after a specimen was caught in the bay of Genoa. This was given the name *Tetrapturus lessonae*, which was synonymized by subsequent authors with *T. herschelii* Gray or with *T. georgii* Lowe. The type of *T. lessonae* is now preserved in the Museum of Natural History in Genoa; another similar specimen was also caught long ago in the Bay of Genoa and still exists in the Zoological Museum of Turin[3]. Both these fishes are adult and are easily recognizable as *Makaira albida* (Poey). Such identity was suggested by La Monte[4] and is fully confirmed by careful study of all their characters; during a recent visit to Florida I became familiar with *M. albida*. This species is well known in the Atlantic, including its eastern part, where it has been recently observed[5]. It certainly exists in the Mediterranean too, but it seems to be of very rare occurrence in this Sea; maybe it does not reproduce there. It is probable, however, that some records of *T. belone* really belong to *M. albida*. We do not know at present whether the *Tetrapturus* living in the Atlantic (for example, Florida) are the same species as those of the Mediterranean. It would appear unlikely that such widespread pelagic fishes as Istiophorids should include a species restricted to the Mediterranean. As new investigations on these huge fishes are carried out, it becomes more and more evident how far we still are from adequate knowledge of them. *T. belone* and *M. albida* are very similar, both morphologically and biologically, and I do not feel certain that they deserve to be generically separated (rather than included in *Makaira* Lac., 1803, which has priority over *Tetrapturus* Raf., 1810). I would entirely reject their placing in different families, Istiophoridae (*Tetrapturus*, *Istiophorus*) and Makairidae (*Makaira*), which is the procedure adopted by Bertin and Arambourg[6].

Ben Tuvia[7] reported a young *Istiophorus gladius* (Brouss.) from Haifa (Israel) and supposed it to be an immigrant from the Red Sea through the Suez Canal. I know no other Mediterranean records of this easily identifiable genus. It is to be looked for chiefly in the southern parts of this Sea, not only eastward, but also in the western section, where the occasional occurrence of the Atlantic form (*I. americanus* Cuv. Val.), that exists along the coasts of Africa and Europe, is quite possible. *Makaira albida* is to be expected in the whole western basin of the Mediterranean. An expert student of Istiophorids, Col. J. K. Howard (Marine Laboratory, University of Miami), recently told me that this fish is sometimes caught off Ceuta and La Linea.

In conclusion, the Mediterranean fauna certainly includes at least three species of Istiophoridae: *Tetrapturus* (or perhaps *Makaira* ?) *belone* Raf. (the commonest, known for a long time in the Mediterranean only), *Makaira albida* (Poey) (Atlantic; recorded from the Bay of Genoa), *Istiophorus gladius* (Brouss.) (Indo-West Pacific; recorded from Haifa).

As stated above, further researches are needed. With the co-operation of some ichthyologists from the United States, we hope to improve the knowledge of the Mediterranean Istiophorids and to reach a better understanding of their relationships with the species living in the Atlantic.

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Development of the Polychaete *Fabricia sabella* (Ehr)

A striking feature of the rock shores of the southern coast of Northumberland is the presence of extensive silt patches which lie on top of the rock platforms in the intertidal area. These patches are occupied characteristically by dense populations of the sabellid worm, *Fabricia sabella* (Ehr).

While engaged on an ecological study of this annelid the opportunity was taken to investigate its breeding. Rasmussen has been quoted by Thorson[1] to have expressed the opinion that the reproduction of this species is non-pelagic. A study of the Northumberland population has shown that not only is the development non-pelagic but also that the entire larval development takes place within the tube of the adult female.

When the tubes of adult females collected in May were dissected open the presence was observed of small batches of eggs and larvæ in various stages of development situated in small dilations of the tube, each batch being covered with a mucus envelope. Up to ten batches could be found in a single tube with 1–7 individuals in each batch. The batches were distributed along the length of the tube from the

Table 1

Tube No.	Top of tube 1	← Batch No. → 2	3	Bottom of tube 4
1	4 Stage I	6 Stage IV	Larvæ hatched, mucus envelope left	4 Stage V
2	4 Stage I	5 Stage III–IV	2 Disintegrating	
3	3 Stage I	4 Stage III–IV	2 Stage I	
4	7 Stage I			
5	4 Stage III–IV	3 Stage IV	5 Stage V	

bottom to the top and all stages of development from fertilized egg to active pre-adult worm could be observed.

At no stage was any swimming larva observed, and in fact the early larvæ appear to be atrochous. Five arbitrary stages of external characters have been recognized in the development: (1) large yolky uncleaved egg 150–200μ; (2), cleavage stages to gastrula; (3), early hatched larvæ—pyriform in shape and without obvious external features (250–300μ); (4), a more elongated form (350μ) with 2 velum-like branchial lobes and incipient ventral lip, 8 chætigers and both anterior and posterior eyes; (5), the final stage has nine of the adult eleven chætigerous segments, the branchiæ are well developed and the worm is crawling actively. At this stage the young worms apparently leave the tube of the adult. Table 1 gives the data derived from the tubes of five adult females and shows the number of batches in a tube, the number of individuals per batch, and the arbitrary stages of the individual's development.

The information regarding reproduction in the Sabellidae has been summarized by Wilson[2]. The most similar development to *Fabricia* appears to be *Dasychone lucullana* Delle Chiaje, the eggs of which are enclosed in a mucus sac shaped like a thick ring around the mouth of the tube of the parent. Although in this case the early larva has a strong prototroch, it is not clear from the account of Claparède and Mecznikow[3] whether a swimming larva occurs.

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A Spartinetum at Baldoyle in Ireland

In 1870, a species of *Spartina*, known later as *S. townsendii* (H. and J. Groves), was collected from the mud-flats near Southampton. It was growing in a mixed community of *S. alterniflora* and *S. maritima* (then *S. stricta*). It resembled the latter species so strongly that it was labelled '*S. stricta*', and was overlooked until 1878, when Townsend recorded the species officially in his *Flora of Hampshire*[1]. It was assumed generally that the intermediate *S. townsendii* was a hybrid between *S. alterniflora* and *S. stricta*; for example, Stapf[2] stated "the evidence is circumstantial but strong, resting upon many of the characters being intermediate between those of its presumed parents, *its pronounced instability, and its varying fertility*". [The italics are ours.] In 1930, Huskins[3] furnished cytological evidence favouring this assumption when he found that *S. townsendii* had a mitotic chromosome count of $2n = 126$, while *S. alterniflora* and *S. maritima* have $2n = 70$ and $2n = 56$, respectively.

From that time until recently, it was accepted that all *S. townsendii* growing at Southampton would be amphidiploid. In 1956, however, male-sterile plants were found at several localities near Southampton[4]. They resembled the type specimens of *S. townsendii*, and had a chromosome count of $2n = c.\ 63$ (ref. 5). Thus it was established that the primary F_1 hybrid was still in existence in the area, and that it was sterile in all cases examined. It was suggested that this sterile F_1 hybrid was *S. townsendii*, that the seed-bearing *Spartina* had been incorrectly identified with *S. townsendii*, and that this seed-bearing species was temporarily nameless.

The only species of *Spartina* known in Ireland[6] was introduced as cuttings in 1925[7]. It was planted soon afterwards in mud in the Baldoyle Estuary some eight miles north-east of Dublin, but was not officially recorded from that area until 1937[8]. In November, 1958, an investigation was begun of the extensive and spreading meadow of *Spartina* which now fills part of the estuary. The following interesting facts have already emerged from the investigation.

Spartina plants show the "pronounced instability" and "varying fertility" recorded by Stapf[2]. They vary in stature from short, stunted ones closely resembling *S. maritima*, to tall vigorous ones which exceed *S. alterniflora* in height by as much as 1 ft. This range in stature is wider than that usually ascribed to the amphidiploid *S. alterniflora* × *S. maritima*. It is paralleled by a correspondingly wide variation in the inflorescence, where one spike is very frequent on stunted plants, and up to sixteen have been found on tall, vigorous ones. Lush growth is typical of older *Spartina* meadow where the plant is growing in foul-smelling, deep, soft mud, and is clearly visible at full tide; shorter stature is characteristic of meadow where the plant is growing in more sandy mud, and is completely covered for some time by every tide; finally, extreme stunting and a red colour characterize small clones growing in sandy areas bordering the muddy reaches of the estuary; they are covered by every tide. Similar stunting has been observed by Chater and Jones[9] for *Spartina* plants growing in a sandy habitat in the Dovey Estuary.

Male sterility is quite common. In any clone, inflorescences vary from completely male-sterile to normal. The term 'male-sterile' is used here to describe inflorescences in which the anthers fail to emerge, or if they do emerge, fail to dehisce, and in which pollen is variable in size and content. 'Normal' inflorescences are those in which abnormalities were not observed. Seeds have been found in inflorescences

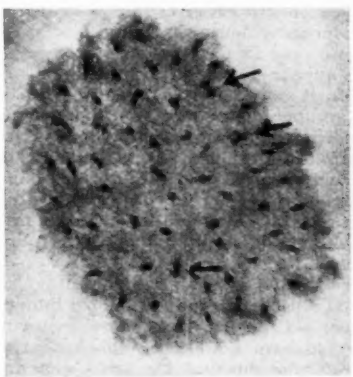

Fig. 1. The three quadrivalents are marked with arrows

of all types and in all parts of the marsh, but so far, 80 per cent of the inflorescences contained 0–5 seeds only. It was not possible to detect the presence of seed in spikelets except by dissection or touch. The latter method was used. All seeds were normal, and 100 per cent germination was obtained. The chromosome number of all plants examined was $2n = 126$ (Fig. 1). Three quadrivalents and 57 bivalents are present in the cell illustrated, and quadrivalents are common at meiotic metaphase. Meiotic abnormalities were observed in some pollen mother cells of all anthers examined, multivalents, asynapsis and lagging chromosomes being the most usual.

Male sterility has been used to distinguish the diploid hybrid *S. alterniflora* × *S. maritima* with $2n = 63$, from the tetraploid with $2n = 126$, but in the Baldoyle Estuary male sterility is quite frequent in clones with $2n = 126$. It is suggested, therefore, that until some clear-cut morphological distinction is observed, the name *S. townsendii* (H. and J. Groves) is equally applicable to both known cytotypes.

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Influence of Date of Flushing on Flowering in *Pinus banksiana*

Numerous attempts have been made to relate the year-to-year variability in flowering of forest trees to seasonal fluctuations in weather conditions which, in turn, are believed to influence the internal physiological processes regulating flower initiation[1]. The pronounced effect of photoperiod on flowering, noted in many herbaceous species, appears to be lacking in forest trees, and observations indicate that most trees are photoperiodically day-neutral[2,3]. However, until more definitive experiments have been undertaken, photoperiod cannot be ruled out as a factor influencing flower formation.

Since weather factors, primarily temperature and rainfall, appear to exert an influence on flower-formation, it may be that favourable weather conditions and/or photoperiod must be synchronized with a particular developmental stage of the bud for flower primordia to form. It might be assumed that such synchronization would be thrown out of phase by an exceptionally early or late spring which would shift the receptive stage of development into a more favourable or unfavourable set of environmental conditions.

To test this possibility, an exploratory study was conducted with jack pine (*Pinus banksiana* Lamb.). Three-year-old potted trees were overwintered in outdoor cold-frames, and the following spring (1959), prior to normal flushing, the trees were transferred to a cold-storage room. On the dates indicated in Table 1, lots of 30 trees each were brought into a greenhouse maintained at minimum temperatures of 60° F. (night) and 72° F. (day); late April and May day-temperatures usually exceeded this minimum in the greenhouse. The plants were exposed to the natural photoperiod and were watered daily. Shortly after the last group (May 18) was removed from cold storage, the trees of all treatments were transplanted in nursery beds and exposed to prevailing environmental conditions. The controls were 3-year-old trees transplanted directly in the nursery beds 1 year prior to the treated plants and were therefore subjected to a natural environment during the spring of 1959. At the time of floral induction, the treated trees were in their fourth, and the controls in their fifth, growing season.

Table 1. Flowering Response of Young Jack Pine Trees for Varying Dates of Flushing

Treatment date	Total No. of trees	No. of trees flowering				Total No. of strobili	
		Male	Per cent	Female	Per cent	Male†	Female
4/ 6/59	28*	5	17·8	22	78·6	199	40
4/20/59	30	12	40·0	20	66·7	482	30
5/ 4/59	30	—	—	19	63·3	—	33
5/18/59	30	1	3·3	15	50·0	1	23
Control‡	123	—	—	70	56·9	—	134

* Excluding two trees that died in the nursery.
† Male strobili produced in clusters of aments varying from 1 to 32 in number.
‡ Control, 5-year-old trees; treated groups, 4-year-old trees.

The results, shown in Table 1, are based on strobili counts made in late May and early June, 1960. The results show a marked superiority of the April 20 date for staminate, and the April 6 date for pistillate, strobili-formation. This differential response is no doubt due to the location and the sequential initiation of the flower primordia of the two sexes. Male strobili occur on the basal portion of the new shoot of older lateral branches whereas the female strobili, at least in young jack pine, occur primarily on the apical end of the main shoot contiguous with the lateral branch buds. Jack pine is multinodal in the bud[4], and occasional female strobili may be found at the secondary whorl positions of the new shoot as well as at the apical end of the newly formed laterals. These differences in position, which are characteristic of the genus *Pinus*[5], indicate that the staminate flowers may be laid down well in advance of the pistillate flowers, as shown for other members of the hard pine group[6–9]. Although phenological data are not available for jack pine, information for other species of *Pinus* indicates that the time differential between the initiation of flower primordia for the two sexes may be in excess of several weeks.

Several environmental factors may contribute to the variable flowering response between treatments, the most obvious of which are photoperiod and temperature. Each set of buds would be subjected to a different régime of photoperiod and temperature from the buds of the previous and subsequent treatments as they attained the stage of development at which flower primordia were initiated. Photoperiod varies uniformly and can be readily evaluated. Temperature and temperature-photoperiod interactions, however, cannot be readily evaluated under natural conditions as wide fluctuations occur throughout the growing season. The possibility must also be considered that neither photoperiod nor temperature exerts a direct influence on flower initiation and that the differential response to treatment may be due to the length of the growing season available for bud-development. Data have been presented indicating that source of seed also influences significantly the

flowering response of young jack pine[9]. Because of the preliminary nature of the present investigation, definite conclusions cannot be drawn, but continuation of the work under more exacting conditions may permit critical evaluation of the influencing factors.

The data obtained nevertheless suggest that the seasonal fluctuations in date of flushing may be one of the factors responsible for variable flowering behaviour. Such fluctuations would place the dates of male and female flower initiation in different sets of environmental conditions and these would vary from year to year. Moreover, due to the vagaries of weather conditions, the same date of flushing in subsequent years may not necessarily evoke the same flowering response.

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Rootstock Influence on the Flowering of Seedling Apples

THERE has long been divergence of opinion as to whether or not the flowering of seedling trees, especially pome fruits, could be hastened by cultural practices or through environmental changes. At the California Institute of Technology, it was found with *Pinus* that the 'juvenile' or initial non-flowering period of the seedling's life could be shortened following artificial lengthening of the period of summer growth and prolonged winter dormancy[1]. In apples, any treatment that restricts or retards growth has been held by some to delay the onset of reproductive ability, while others have held the contrary view[2,3].

Experience at East Malling has long suggested that seedling material budded on the dwarfing Malling (*M.*) IX (Jaune de Metz) rootstock fruited distinctly earlier than could be expected of seedlings on their own roots[4]. No evidence was available as to whether this effect was attributable to the individual characteristics of the *M.*IX rootstock as such or directly to its dwarfing and precocious character.

In the course of a very considerable programme of crossing and selfing to produce, directly or through later back-crossing, apples combining eating quality with disease resistance and with the character of late flowering as a means of avoidance of spring frost damage, hundreds of seedlings selected from large families on the basis of late leafing and disease resistance have been retained on their own roots, to provide material for future propagation. A bud from each of these seedlings, usually taken in the seedling's second year, has also been grown on one or other of two dwarfing rootstocks, *M.*IX and 3431, the latter having the parentage *M.*XIII × *M.*IX. Normal *T* budding on 18-month old bedded layers was practised.

Table 1

Family No.	No. of seedlings	Trees on their own roots: Flowering at five years	Flowering at six years	Flowering at seven years	Trees budded on *M.*IX: Flowering at three years	Flowering at four years	Flowering at five years
1	138	0	13	24	11	93	107
2	126	0	3	12	29	83	95
3	13	0	0	2	1	6	10
4	7	0	0	2	1	6	6
5	164	0	1	1	5	47	41
6	15	0	1	0	0	5	6
7	139	0	2	5	1	24	14
Totals	602	0	20	46	48	264	279

Seven families were raised in 1954 and buds from 602 selected seedlings were worked on *M.*IX rootstocks in the summer of 1956. The numbers that flowered are given in Table 1.

A further seven families were raised in 1955 and buds from 302 selected seedlings were worked on rootstock 3431. The numbers that flowered are shown in Table 2.

Table 2

Family No.	No. of seedlings	On their own roots: Flowering at five years	Flowering at six years	Budded on 3431: Flowering at three years	Flowering at four years
8	169	11	19	37	67
9	36	0	4	5	12
10	39	0	3	7	19
11	20	2	2	1	8
12	2	0	1	1	1
13	9	0	0	0	3
14	27	0	0	0	4
Totals	302	13	29	51	114

Thus, on *M.*IX, 43 per cent had flowered in the sixth year from germination as against 3 per cent on their own roots. On rootstock 3431, 38 per cent had flowered in six years from the seed as against 9 per cent on their own roots.

As neither the families nor the period of experiment were identical, it is difficult to compare the effects of *M.*IX and rootstock 3431 on such limited data. It may not, however, be out of place to note that 3431 is more dwarfing than *M.*IX[5]. A more comprehensive experiment is needed if this clearly demonstrated effect of dwarfing rootstocks in reducing the 'juvenile' period is to be related more clearly to the degree of 'dwarfing' characteristic of the individual rootstock. Further, it must not be forgotten that, while *M.*XIII is markedly more vigorous, *M.*IX is one of the parents of 3431. The possibility of a specific heritable attribute is therefore not excluded.

It would seem[4,6] that the effect reported here is not to be related either to the relative mass or to the physiological age of the stock, since working small seedlings on large trees of long-established commercial varieties of apples had no effect on shortening the 'juvenile' period. The work reported here supports the view that its length, while clearly susceptible to influence by dwarfing rootstocks, also varies markedly from seedling to seedling and from family to family.

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Replacement of an Alternating Temperature Requirement for Germination by Gibberellic Acid

IN many species of plants, seed-germination does not take place readily except under special conditions. A barrier to germination may be imposed by the seed coats or other coverings, by the presence of germina-

tion inhibitors in some part of the seed, by morphological or physiological immaturity of the embryo, by the need for light or for its absence, or by the need for alternating temperatures during germination[1]. Chemical substances can in some cases substitute for specific environmental factors in effecting seed germination. Nitrates, for example, promote the germination of some light-requiring seeds in the dark[1], and the results of recent work indicate that the plant hormone gibberellic acid is particularly effective in overcoming several types of germination inhibition. In the germination of 'Grand Rapids' lettuce seeds, the primary light-requirement[2,3] and the light-requirement induced by high temperature or dark-osmotic treatments[3] can be replaced by gibberellic acid. Gibberellic acid overcomes the inhibiting effect of coumarin on lettuce seed germination[4] and of endogenous germination inhibitors present in *Avena fatua*[5] and *Betula* spp.[6] seeds. Kallio and Piiroinen[7] found that gibberellic acid has a marked stimulating effect on the germination of seeds of 8 species, including *Gentiana nivalis* and *Geranium sylvaticum*; their experiments were conducted in the light at 24°.

We have examined the effect of gibberellic acid on another type of germination inhibition, namely, that caused by a requirement for alternating temperatures. A series of experiments has been conducted with *Asclepias fruticosa* seeds, which do not germinate at constant temperatures ranging from 10 to 35° C., but only at alternating temperatures[8]. Repeated cycles of alternating temperatures are not essential; if the seeds are kept at 18° C. for 7 days, transferred to 35° C. for 4 hr. and replaced at 18° C., 80–100 per cent germination is obtained within 3 days after the return to the lower temperature[8]. These seeds are not light-sensitive.

Table 1. Effect of Gibberellic Acid on the Germination of *Asclepias fruticosa* Seeds

50 seeds per treatment. No germination took place before treatment with gibberellic acid

Gibberellic acid (p.p.m.)	Percentage germination after 2 days	4 days	6 days	8 days	14 days
0	2	4	4	4	4
1	2	4	4	10	20
10	16	30	46	56	72
50	12	50	74	82	96
100	12	64	92	94	96
500	6	16	26	34	38
1,000	0	0	0	6	10

The effect of gibberellic acid on the germination of *Asclepias fruticosa* seeds was studied at a constant temperature of 18° C. in the light. The seeds were placed on moist filter paper in Petri dishes. After 3 days, the seeds were pricked by passing a fine needle through the seed coats, endosperm layers and cotyledons, and were transferred to Petri dishes containing filter papers moistened with gibberellic acid solutions at concentrations ranging from 0 to 1,000 p.p.m. The results of a typical experiment are presented in Table 1. Rapid, almost complete, germination was obtained when the seeds were treated with 50 or 100 p.p.m. gibberellic acid. Concentrations of gibberellic acid lower than 50 p.p.m. were less effective in promoting germination and concentrations higher than 100 p.p.m. were inhibitory. Gibberellic acid was completely without effect if the seeds were not pricked, which indicates that the seed coats and/or the endosperm layers may be impermeable to gibberellic acid. In this respect, *Asclepias fruticosa* seeds are similar to those of *Geranium sylvaticum*[7], which do not respond to gibberellic acid unless they are first scarified with sulphuric acid.

These results show that gibberellic acid is highly effective in replacing the need for alternating temperatures in the germination of *Asclepias fruticosa* seeds. Experiments are in progress with the seeds of *Argemone mexicana*, which normally germinate only if subjected to repeated cycles of alternating temperatures in the presence of potassium nitrate, and the preliminary results indicate that gibberellic acid promotes rapid seed germination at a constant temperature in the absence of nitrate. It will be of interest to determine how general the promotive effect of gibberellic acid on the germination of seeds having special requirements is. The effect of gibberellic acid on dark-germinators, for example, does not seem to have been investigated.

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Relationship between 3,4-Dihydroxyphenylethylamine Content and the Genome, *acuminata*

The major polyphenoloxidase substrate responsible for the blackening of the fruit of the banana has been identified[1] already as 3,4-dihydroxyphenylethylamine. Although the original work was carried out using material of the clones Gros Michel and Mysore, a range of clonal varieties was investigated afterwards to determine whether this substrate was responsible for blackening in other clones or whether other phenolic compounds were implicated in the blackening reaction.

It has been found that in all clones of the edible banana investigated the major polyphenoloxidase substrate is 3,4-dihydroxyphenylethylamine and furthermore that a positive correlation could be shown between the content of 3,4-dihydroxyphenylethylamine and the extent of the contribution of the genome *acuminata* to the genotype.

It is now recognized[2] that the majority of the edible bananas had their origin in only two wild species, *Musa acuminata* and *M. balbisiana*. Simmonds and Shepherd[3], using a scoring method, were able to establish the relative contribution of the two wild-species to any given clone. The genetic composition of each of the clones investigated is shown in Table 1. All plant material for the investigation was obtained from the collection of banana plantings at the Imperial College of Tropical Agriculture, Trinidad. Earlier work on the distribution of 3,4-dihydroxyphenylethylamine in the banana indicated that although

Table 1. CONTENT OF 3,4-DIHYDROXYPHENYLETHYLAMINE IN PREPARATIONS OF CLONES OF DEFINED GENOTYPE

Clone	Genotype (ref. 2)	Dihydroxyphenylethylamine content*
Kelantan	*AA*	+ + + +
Ceylon	*BB*	+
Pancha Bontha Bathees	*ABB*	+ + +
Nendra Padaththi	*AAB*	+ + +
Red	*AAA*	+ + +
Calcutta	*AA*	+ + +
SH 62	*AB*	+ +
Key Mannan	*ABB*	+
Guindy	*AB*	+
Mporo Moka	*AAB*	+ +
Bluggoe	*ABB*	+
Tiparot	*ABBB*	+
Rajah	*AAA*	+ + +
Gitigi	*AAA*	+ + + +
Ykonjwanshansha	*AAB*	+ + +
Dwarf plantain	*AAB*	+ + +

* Amounts assessed by size and intensity of the corresponding *p*-nitroaniline spots on two-dimensional chromatograms.
A, Genome, *acuminata*; *B*, genome, *balbisiana*.

present in small amounts in the pulp, larger amounts were present in the skin of the fruit. Extracts of the skin were therefore used for the comparative investigations described here.

Methanolic hydrochloric acid extracts of skin tissue obtained from a range of clones were prepared by methods described before. 0·02 ml. of each extract was applied to duplicate paper chromatograms (Whatman No. 1) and subjected to two-dimensional development with butanol/acetic acid/water (40: 10: 50) followed by 2 per cent acetic acid. One member of each pair of duplicate chromatograms was sprayed with *p*-nitroaniline reagent and the other with a banana polyphenoloxidase preparation. After incubation of the enzyme-sprayed chromatogram in a humid chamber, brown colorations developed in the position corresponding to that of 3,4-dihydroxyphenylethylamine. The size and intensity of the spots obtained with *p*-nitroaniline reagent in the position were proportional.

The results which are shown in Table 1 indicate that the content of 3,4-dihydroxyphenylethylamine is determined by the extent of the contribution of the *acuminata* genome to the genotype.

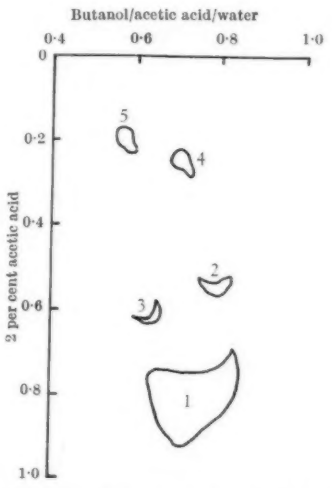

Fig. 1. Composite diagram showing the position of fruit phenolics of banana cultivars. 1, 3,4-dihydroxyphenylethylamine; 2–5, unknown minor phenolic constituents. (Colorations with *p*-nitroaniline: 1, purple; 2 and 3, red; 4 and 5, brown. Ultra-violet fluorescence: 2, white; 3, green; 1, 4 and 5, non-fluorescent)

Two-dimensional chromatography (Fig. 1) showed that 3,4-dihydroxyphenylethylamine is the only major phenolic present in the fruit investigated. Although a number of minor phenolic constituents were detected, these were present in trace quantities only and clearly did not contribute to any appreciable extent to the blackening of the fruit. Clonal differences were almost wholly quantitative, the same phenolics being present in all the clones to a varying degree. The major source of variation was (as noted in Table 1) the content of 3,4-dihydroxyphenylethylamine, the amounts present ranging fron trace quantities in *M. balbisiana* varieties to very high levels in the *M. acuminata* cultivars.

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CYTOLOGY

Cytology and Origin of the Dwarf Coconut Palm

DISTINCT varieties of dwarf coconut (*Cocos nucifera* L.) occur in Malaya, Philippines, Fiji Islands, Viet Nam, Ceylon and India. These varieties grow to a height of 15–30 ft. compared with more than 50 ft. among normal tall types and need only 3–5 years to commence bearing in contrast to 8–10 years taken by the tall strains. While the tall varieties are largely obligatorily cross-pollinated, the dwarfs usually undergo self-pollination owing to the overlapping of the female and male phases in the same inflorescence. As a result, many dwarf strains yield homogeneous progenies and they can be used with advantage for identifying the most pre-potent males[1]. The poor vigour and copra quality of the dwarf palms, however, render them unsuitable for cultivation on a plantation scale[2]. In addition to the typical tall and dwarf palms, various intermediate categories occur in Nature and have also been obtained by crossing the tall and dwarf strains[3].

Several semi-tall and dwarf varieties of coconut show 15–35 per cent pollen sterility, as judged by a tendency to become stained with aceto-carmine. The tall varieties, on the other hand, contain less than 5 per cent of sterile pollen[4]. A detailed study of meiosis in microsporocytes was hence undertaken in two tall, one semi-tall and three dwarf palms. While meiosis was always regular in the tall plants, the following abnormalities occurred in several cells in the others: (*a*) one quadrivalent at diakinesis and metaphase I, suggesting heterozygosity for a reciprocal translocation; (*b*) a dicentric bridge and an acentric fragment at anaphase I indicating heterozygosity for an inversion; (*c*) nucleoli which persist at metaphase and anaphase I; (*d*) pollen mother cells with varying chromosome numbers; (*e*) lagging chromosomes at the first and second anaphase and (*f*) sporads with varying number of spores. There was also a significant reduction in chiasma-frequency in Apricot (semi-tall) and Green (dwarf), as compared with the two tall varieties. The percentage of cells with abnormalities at different stages of meiosis in the varieties examined is given in Table 1.

Table 1. Meiosis in Some Coconut Varieties

Variety	Type	Percentage of cells with abnormalities at Metaphase I	Anaphase I	Second division	Sporad
Laccadive ordinary	Tall	0	0	0	0·8
Local type	Tall	0	0	0	0
Apricot	Semi-tall	20·4	40·1	24·7	29·2
Dwarf red	Dwarf	4·0	23·6	8·6	7·9
Dwarf green	Dwarf	0	5·1	2·9	4·3
Dwarf yellow	Dwarf	0	4·6	2·2	1·3

The occurrence of various abnormalities and 'chromosome mosaic' cells at meiosis, and a poor development of the endosperm in the dwarf palms, indicate that the efficiency of the chromosome mechanism has been affected in somatic, gametic and endosperm cells. It is probable that the imposition of inbreeding on a normally out-crossed species is responsible for this situation, since it is known that inbreeding upsets the chromosome mechanics in cross-pollinated species[5–7]. Some authors[3,8] have suggested that the dwarf strains of coconut may have originated through a sudden discontinuous mutation from the common tall palm. From the cytological results it seems equally probable that the dwarf coconuts occurring in Nature in different countries may be the products of inbreeding among different tall varieties. This view is also supported by the following. First, the tall and dwarf palms differ in not one but a series of characters all of which seem to lead to reduced vegetative vigour and aberrant cytological behaviour. Secondly, all gradations between the two contrasting categories represented by typical talls and dwarfs occur in Nature. Thirdly, the F_2 progeny of a dwarf × tall cross gave a wide range of segregates and the data did not suggest any simple genetic explanation[2]. The more regular meiosis observed in the dwarf varieties in comparison with the semi-tall type suggests that with progressive inbreeding an equilibrium condition may be reached with regard to cytological behaviour. The same may also be true of vegetative vigour. An initial mutation, if any responsible for the origin of the dwarf coconut might have led to the overlapping of the male and female phases of the inflorescence, thus rendering self-pollination both possible and predominant. However, self-pollination can occur in the tall palms during certain seasons and a mutation facilitating this process is not a prerequisite for inbreeding to occur. Alternatively, a situation analogous to that described by Harland[9] in the maize strains of Trinidad may be responsible for the evolution of stable self-pollinating dwarfs.

Whatever may be the mode of origin of the dwarf coconut, the fact that many geographically distinct and true-breeding dwarf strains exist indicates the possibility that they may be useful both to estimate and exploit heterosis in coconut. Much of the hybridization work carried out on coconut so far relates to crosses between tall and dwarf varieties or among tall strains. The only record of a cross between two geographically distinct dwarfs seems to be that made by Marechal[10] between two dwarf strains from Malaya and the Fiji Islands. The F_1 plants of this cross showed outstanding performance and have been much sought after in Fiji[11]. Harland[1] has suggested the induction of haploidy in coconut for obtaining homozygous genotypes and, if the view that some dwarf coconuts represent stable inbred lines is correct, they may serve the same purpose as the autodiploids derived from haploids. Harland's technique for identifying pre-potent males would be ideal for estimating combining ability in different dwarf × dwarf crosses.

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MICROBIOLOGY

Factor converting Mesophilic into Thermophilic Micro-organisms

In the course of work on thermophilic, aerobic, spore-forming bacteria, we have observed that the thermophilic cells contain, in certain conditions, a principle, which, when added in small quantity to a fresh culture medium, permits an inoculum of a mesophilic organism to grow at elevated temperatures. Moreover, this induced thermophily is carried from one transfer to another for an indefinite period.

Concentrated preparations of this factor produced the effect in quantities of 5 μgm. per ml. of medium. The properties, described here, suggest that the factor is in the nature of a nucleoprotein. We have also observed that it is only present, or at least extractable, during vigorous growth of the thermophilic culture, but not after the exhaustion of nutrient has led to sporulation.

The addition to a medium of an autolysate obtained from *Saccharomyces cerevisiae* also permitted the growth of a mesophilic inoculum at 55°, but this effect appears to differ from that obtained by the homologous factor inasmuch as thermophily cannot be indefinitely maintained without renewed addition of the yeast factor.

The source of the principle is *Bacillus stearothermophilus* No. 194, grown in a medium described elsewhere[1], and supplemented with 1 per cent casein hydrolysate. This medium was inoculated with a stock culture of the thermophilic bacillus and incubated overnight at 55°. This culture, now germinated, was inoculated into fresh basal medium and incubated at 55° for 9–11 hr.; this procedure resulted in a small amount of spore-formation. Incubation for 15 hr. or longer periods, when sporulation had set in, did not yield the principle.

After an incubation period of 9–11 hr., cells were collected in a Sharples refrigerated centrifuge at 50,000 r.p.m. Packed cells were washed by homogenization in a Waring blendor with cold distilled water and again centrifuged. The packed bacilli were lyophilized and stored at 4° C. The converting factor was obtained from the lyophilized bacillus powder by suspension in 0·05 *M* sodium citrate and grinding with glass beads (type 112, Minnesota Mining and Mfg.

Co., U.S.A.) for 10 min. in a Waring blendor at 4° C. The debris was centrifuged off at 2,000 r.p.m.; the remaining cell suspension was further centrifuged at 5,000 r.p.m. for 6 hr. at 4° C. The supernatant was discarded. The residue was extracted twice with 50 ml. of 2 *M* sodium chloride; the transforming factor is not stable in aqueous solution. The extract, diluted with 0·16 *M* sodium chloride, was added in varying amounts to a medium, which was then inoculated with a mesophilic strain of *B. sphaericus*. This mesophil was found to grow at 37° in 0·5 per cent beef extract (Difco) plus 0·5 per cent proteose peptone (Difco) but not at 55°. It now grew at 55° on addition of the converting factor down to dilutions of 5 μgm./ml.; growth is still obtained at 37° with or without addition to the principle.

The converting principle is destroyed by deoxyribonuclease, ribonuclease, pepsin and papain (Worthington Biochemical Corp., U.S.A.), while trypsin, chymotrypsin, and carboxypeptidase do not inactivate it. The principle is non-dialysable against distilled water, and is unstable in aqueous solution as compared with 2 *M* sodium chloride. This behaviour is similar to that of deoxyribonucleic acid[2].

The factor will stand 1 min. of heating at 100°, but its heat stability is influenced by the rate of cooling; its efficacy will be less diminished if cooling is carried out slowly; for analogous observations on depolymerization of bacterial nucleic acid, see ref. 3.

These findings suggest that the factor is nucleoprotein. Nucleic acid preparations from thermophilic bacteria were not effective. In order to test substances rich in nucleic material, yeast autolysate (Albimi Laboratories, Brooklyn, N.Y.) was tested and a similar transformation of the mesophilic organism was observed[4]. Growth in medium containing yeast autolysate was required to maintain thermophily; contrary to this finding, once thermophily was induced with thermophil extract, it was no longer required to maintain thermophily on repeated sub-culturing. Attempts to purify and compare the converting factor from the thermophilic bacteria and from yeast autolysate are now under way. We are also studying the specificity of the effect.

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Influence of adding Salts to the Medium on Counts of Phenol-treated *Escherichia coli* on Membrane Filters

IT has been shown[1] that suspensions of *Escherichia coli* which had been treated with phenol gave lower counts on membrane filters than on the surface of nutrient agar. Many workers[2] have observed that bacteria which have been exposed to bactericides or heat exhibit a marked sensitivity to the nature of the subculture medium, and both Flett *et al.*[2] and Jacobs and Harris[3] have shown that when ferric chloride was added to the medium it exerted a beneficial effect, demonstrated by longer extinction times than in control media, on the growth of phenol-treated organisms. The latter workers found that the rate and extent of the death of phenol-treated cells of *E. coli* and *Staphylococcus aureus* which occurred in nutrient broth[4] was reduced by the inclusion of 5×10^{-3} *M* of magnesium chloride, and that the inclusion of ferric chloride and other metal chlorides in nutrient agar gave surface-viable counts of such cells greater than were obtained on nutrient agar without metals[5]. In view of the present widespread use of membrane filters, and suggestions that these filters should be used to recover damaged cells from a variety of harmful environments, for example, in sterility tests, it was thought useful to examine the effect of adding salts to the nutrient broth on which the membranes are incubated.

Chlorides of iron, chromium, calcium, magnesium and magnanese were added to the nutrient broth ('Oxoid *CM* 15', 16 gm./l.) before sterilization to give concentrations of 0·0037, 0·00185, 0·000925, 0·00037, and 0·000185 *M*. The method of counting has been described in ref. 1; ten drops of a diluted suspension were placed on the surface of the membrane and counts on membranes using media containing added salts were compared with those obtained on a control nutrient broth, without salts. In this work only one strain of *E. coli* was used (strain *A* of Harris and Richards[1]), since two of the strains used in the earlier work were known to behave similarly when salts were added to nutrient agar.

The counts of untreated cells did not differ significantly from those on the control whatever the nature or concentration of the cation used, and the results are not presented here. Table 1 gives the mean percentage counts from phenol-treated suspensions in numerous trials at each concentration of the cations, and the significance of the results, given by the analysis of variance test, is indicated also. In contrast to untreated cells, counts of phenol-treated cells were influenced markedly by the presence of salts. However, only iron influenced favourably the counts from treated suspensions at all the concentrations used: except at 0·00037 *M* of chromium and 0·000925 *M* of calcium and magnesium, with which counts did not differ significantly from the control, these three cations also gave favourable responses. Manganese gave variable results and counts were greater and less than on the control with equal frequency. The most favourable of the concentrations tested was 0·00185 *M* with all the cations, and the improvement in counts was most marked with chromium and iron.

Possible mechanisms for the beneficial action of salts have been discussed by Jacobs and Harris[4].

Table 1. MEAN RELATIVE MEMBRANE FILTER COUNTS OF PHENOL-TREATED *E. coli* ON MEDIA CONTAINING VARIOUS CONCENTRATIONS OF A RANGE OF CATIONS

Molarity of ion (× 10^6)	Mean relative viable count* on				
	Iron	Chromium	Calcium	Magnesium	Manganese
3,700	160	154	152	134	54§
1,850	841	1,421	364	478	208
925	221	179	100†	99†	41§
370	174	159†	167	140	136†
185	166	183	165	180	157

* Relative to nutrient broth without added salt as 100.
† No significant difference from control; § significantly less than control, all others significantly greater than control, all at the 5 per cent level at least.

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They suggested that substances in the medium which are toxic to damaged cells could be removed by adsorption on metal precipitates or by co-precipitation with them, and that cations which are not precipitated could reduce the permeability of the damaged cell membrane and so reduce the inward diffusion of such toxic substances and the leakage from the cell of vital metabolites[6,7]. The protective influence of metallic cations, especially those of high valency, against the action of phenol has been observed in ref. 7.

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Enzymic Inactivation of Trichothecin and Crotocin

Work on the inhibitory action of trichothecin[1] and crotocin (substance *T*, ref. 2) on the inductive amylase synthesis of *Penicillium chrysogenum*[3] suggested that these antibiotics may undergo enzymic inactivation in certain circumstances. The possibility of such an enzymic inactivation was first recognized by Darpoux and Faivre-Amiot[4].

Penicillium chrysogenum was grown on a synthetic medium as described previously[5]. Cultures for the experiments were prepared by inoculating 200 ml. synthetic medium with 10 ml. of a 72-hr.-old vegetative mycelium suspension. The fate of crotocin added was examined by two different methods. First, the antibiotic was added in a 1 mgm./ml. single dose after 48 hr. incubation. The level of the antibiotic in such a culture remained unchanged for a minimum of 24 hr. as revealed by the Bradler[6] agar-diffusion method. In the second series of experiments 10 μgm./ml. crotocin was added after 40 hr. and this was followed by the addition of 1 mgm./ml. of the same antibiotic after 48 hr. incubation. Under these experimental conditions a 70 per cent decrease in the antibiotic activity of crotocin was observed in 5 hr.

This phenomenon suggested the possible presence of a crotocin-inactivating enzyme in cultures pretreated with a low dose of the antibiotic. The enzyme appeared to be bound to the mycelia as the supernatant was inactive, while 1 gm. of washed mycelium (calculated on dry material) inactivated an average of 40 mgm. crotocin per hr. The preliminary examinations carried out up to now indicated that the enzyme was probably bound to the cell-wall of the mycelium.

It is known that trichothecin is an ester of trichothecolon and isocrotonic acid[7]. The alcoholic component of crotocin is very similar, though not identical, to that of trichothecolon. It seemed plausible that the decrease of the biological activity was a result of enzymic cleavage of the ester linkage. The validity of this assumption was later confirmed by isolating and chromatographing the split products.

After inactivation of crotocin by washed mycelia the mixture was filtered and the fluid phase was saturated with sodium chloride and extracted with chloroform at *p*H 10 (ref. 8). The extract was evaporated and the oily residue crystallized from benzene. The isolated substance had a melting point of 155·5° C., identical with the melting point of the alcoholic component obtained by alkaline hydrolysis of crotocin. Mixed melting points of the two materials obtained by the two different methods did not exhibit any depression. The acid component was identified by paper chromatography.

According to the above observations crotocin if applied in small quantities can act as an inductor. The enzyme thus induced is able to hydrolyse the ester linkage in the antibiotic which destroys its biological activity. On the addition of a single large dose of crotocin no inactivation of the antibiotic activity could be observed since this quantity inhibited also the synthesis of the inductive enzyme. Crotocin might be replaced by trichothecin, both as an inductor and a substrate.

Other fungi such as *Penicillium urticae*, *Aspergillus parasiticus* and *Aspergillus oryzae* failed to exhibit similar action. *Aspergillus niger*, however, when tested under the same experimental conditions, had an effect similar to that observed for *Penicillium chrysogenum*.

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Detection of Gibberellic Acid in *Azotobacter* Cultures

It is known that *Azotobacter* cultures can affect the germination of seeds and growth of plants. It is assumed that this influence is due to the presence of physiologically active substances and vitamins in the cultures. Actually, indole-3-acetic acid has been detected in *Azotobacter* cultures[1,2]. In a previous paper[2] the detection of another growth-active compound, in addition to indole-3-acetic acid, was described. More details about this compound, which appeared to be gibberellic acid, are presented here.

Azotobacter chroococcum was grown in Burk's solution containing traces of molybdenum and boron, the same procedure as that used for growth of cultures when isolating indole derivatives[2]. The medium did not contain calcium carbonate, however. After ten days of incubation the culture was centrifuged, the supernatant evaporated *in vacuo* to a tenth of its original volume, made acid with 1 *N* hydrochloric acid (to *p*H 2·8–3·0) and extracted with *n*-butanol[3] or

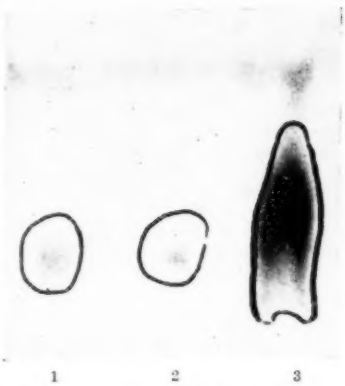

Fig. 1. Chromatogram of gibberellic acid from culture of *Azotobacter chroococcum*. 1 and 3, various concentrations of *n*-butanol extract; 2, 50 μgm. of gibberellic acid

peroxide-free ether[2]. Individual extracts were placed on Whatman No. 1 filter paper and chromatograms developed in four systems: butyl acetate saturated with water[3], benzene / pyridine / 3 *M* - ammonium hydroxide (3 : 6 : 1)[4], benzene/acetic acid/water (10 : 2·5 : 5)[5] and *n*-butanol/formic acid/water (4 : 1 : 5). Best separation of indole-3-acetic acid from gibberellic acid was obtained in butyl acetate saturated with water. Gibberellic acid remained at the start ($R_F = 0·1$) while indole-3-acetic acid moved nearer the front ($R_F = 0·8$).

The last-named system, however, has the drawback of forming 'tails' behind the spots of both acids. From the point of view of shape and sharpness the system with acetic or formic acid is more advantageous as dissociations of indole-3-acetic acid and gibberellic acid are suppressed and sharp round spots are obtained. In the formic acid system the R_F values of the two growth substances are very close, which represents a handicap in elution of the spots from the chromatogram and in biological testing of the eluate.

Detection of gibberellic acid on chromatograms was carried out with 3 per cent sulphuric acid in methanol followed by heating to 60° C. for 5 min.[4], or with 0·5 per cent aqueous potassium permanganate[5]. By adding 0·05 per cent ferric chloride to the methanolic sulphuric acid the reagent can be used for detecting both indole-3-acetic acid and gibberellic acid. Indole-3-acetic acid gives a violet-red colour and displays no ultra-violet fluorescence while gibberellic acid shows a blue-green ultra-violet fluorescence but gives no colour reaction in visible light.

The position of the spot of the substance tested which gives a colour reaction with both reagents corresponds in all the four systems used to the R_F of a standard solution of gibberellic acid. Fig. 1 shows a chromatogram developed for 8 hr. in benzene–pyridine–3 *M* ammonium hydroxide (3 : 6 : 1) in the downward direction. We used the system described by Bird and Pugh[5] to demonstrate that the *Azotobacter* strains contain mostly gibberellic acid (gibberellin A_3) rather than gibberellin A_1. The chemical detection of gibberellic acid was confirmed by a biological test. Undetected spots corresponding to gibberellic acid on the chromatograms were eluted with distilled water and a part of the eluate was tested on etiolated sections of maize leaves and mesocotyls, as described by Boyarkin and Dmitriyeva[6]. The test is specific for gibberellins. Indole-3-acetic acid, if present in the solution tested, has a slight inhibitory effect. A positive significant influence of the eluate on longitudinal growth of the maize leaf was established.

The positive influence of the biological test, together with the results obtained by paper partition chromatography technique, indicates the presence of gibberellic acid in *Azotobacter* cultures. The amount of gibberellic acid in the supernatant of a 10-day-old culture varied under the above cultivation conditions between 2 and 5 μgm./ml. In a 17-day-old culture, where the source of carbon had been exhausted, the amount of gibberellic acid rose to 20 μgm./ml. It is assumed that the presence of gibberellic acid together with indole-3-acetic acid in *Azotobacter* cultures can have a connexion with the frequently noted effect of *Azotobacter* cultures or their filtrates on germination of seeds or growth of seedlings.

So far as is known from the literature the presence of gibberellic acid has been demonstrated in cultures of *Fusarium moniliforme*[7] and other *Fusarium* species[8], of some non-identified Actinomycetes[8] and of the soil yeast *Torula pulcherrima*[9]. The finding described here supplies evidence for the existence of gibberellic acid in *Azotobacter chroococcum* Beij. It appears that gibberellic acid is more prevalent among micro-organisms than was believed, even if in lower concentrations than those found in *Fusarium* cultures.

I am indebted to M. Podojil of the Institute of Biology for a gift of a mixture of gibberellin $A_1 + A_3$.

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Nutritional Requirements for the Induced Formation of Malic Enzyme in *Lactobacillus arabinosus* (17-5)

In 1948, Korkes and Ochoa[1] described an enzyme preparation from *Lactobacillus arabinosus* which catalysed the conversion of L-malic acid to lactic acid and carbon dioxide. They called it malic enzyme. The activity of this enzyme was found to be very low in organisms grown on ordinary medium. When malic acid was also included in the medium during growth, the ability of the organisms to break down malic acid was found to have increased about fifty times. They also demonstrated that the presence of the growth medium and glucose was essential to the formation of the enzyme.

The growth medium of *L. arabinosus*, like that of other lactic acid bacteria, is very complex. It contains hydrolysed casein, a mixture of inorganic salts, glucose, and special supplements of amino-acids, vitamins, purines and pyrimidines. Gale has shown[2]

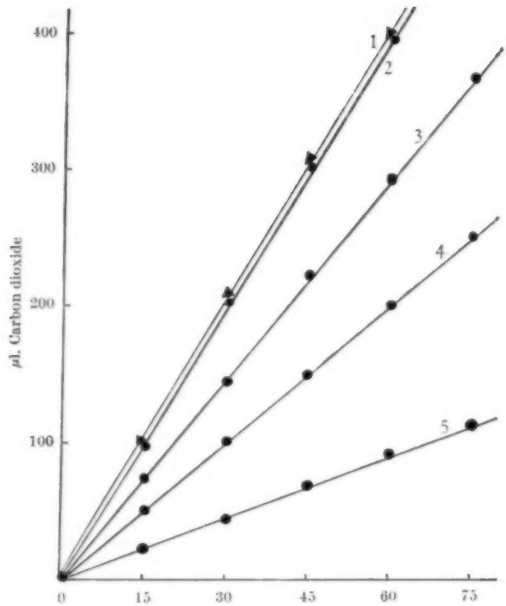

Fig. 1. The effect of nutrition on malic enzyme induction. The samples contained 0·04 *M* L-malic acid, *p*H 6·0, manganese ions, 0·1 *M* phosphate buffer and 5·0 mgm. dry weight cells, suspended in phosphate buffer. Curve 1, complete medium; curve 2, supplement containing eleven amino-acids and a purine/pyrimidine mixture; curve 3, supplement containing eleven amino-acids only; curve 4, supplement containing purine/pyrimidine mixture only; curve 5, control, contained no supplement. In all cases the incubation mixture also contained 1 per cent glucose, manganese ions and phosphate buffer

that the nutritional requirements for optimum growth of organisms may differ from those necessary for the formation of a particular enzyme, and in a recent review. Lichstein[4] has directed further attention to this phenomenon. It was interesting, therefore, to find out which constituents of this complex medium may be more directly involved in the formation of malic enzyme.

The activity of malic enzyme in intact cells of *L. arabinosus* was determined manometrically, by recording the evolution of carbon dioxide from malic acid, at *p*H 6·0 and 30° C. using small Warburg flasks.

In order to separate the requirements of induced enzyme formation from those of growth, it was necessary to use resting cells in these experiments. The organisms were collected from the ordinary growth medium by centrifugation, washed and re-suspended in phosphate buffer (*p*H 6·0). The cell suspensions were incubated for 90 min. with malic acid (adjusted to *p*H 6·0 with sodium hydroxide), glucose, manganese ions and the supplement to be tested. That no net growth occurred during the incubation period was judged from optical density measurements. After the incubation period, chloramphenicol was added to the incubation mixture to prevent the formation of more enzymes. The cells were again separated by centrifuging, resuspended in buffer containing chloramphenicol, and their ability to dissimilate L-malic acid was determined manometrically.

In the initial experiments, the supplement used in the incubation mixture was made up of 5 per cent vitamin-free casamino-acids and a purine/pyrimidine supplement, and the results showed that enzyme formation had been stimulated to the same extent as in the control which contained the complete medium. The investigation was therefore limited mainly to testing the effect of various amino-acids, purines and pyrimidines in promoting the formation of malic enzyme. The vitamin-free casamino-acids were then replaced by a mixture of the 19 amino-acids known to be present in hydrolysed casein. Finally the amino-acids were appropriately deleted and substituted until it was found that a mixture of the following eleven amino-acids could promote malic enzyme synthesis to the same extent as the complete mixture of nineteen acids :

L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, DL-phenylalanine, L-tryptophan, L-tyrosine and DL-valine, the final concentration of each amino-acid in the supplement being 25 mgm. per 100 ml. The purine/pyrimidine supplement consisted of adenine sulphate, guanine, thymine and uracil, the final concentration of each base being 10 mgm./100 ml.

When tested separately the amino-acid mixtures proved more effective than the purine/pyrimidine supplement in promoting the synthesis of the malic enzyme in this organism. But only when added together was the enzyme formed to the same extent as in the presence of the complete growth medium (Fig. 1).

It was interesting to find that the vitamin supplement showed no effect in promoting synthesis of the enzyme, especially since a large number of vitamins have to be provided if this organism is to grow successfully in a synthetic medium. Ochoa and others have also demonstrated the inability of cells starved of biotin to form malic enzyme. It is possible, however, that washed suspensions of normal cells contain enough vitamins to permit the formation of this induced enzyme.

This work was carried out with a grant from the Department of Scientific and Industrial Research.

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VIROLOGY

Influence of Tomato Mosaic Virus Infection on the Hollow Fruit Condition of Tomatoes

Infection of tomato plants by tomato mosaic virus (T.M.V.) may have a very considerable influence on the quality of the fruit harvested. It has been demonstrated[1,2] that poor colouring and/or fruit bronzing may follow virus infection.

Hollowness is the term used to describe a condition of the maturing fruit in which the pericarp becomes separated by a cavity from the seed-bearing axial placenta (Fig. 1). This cavity may be either discontinuous or continuous, in the latter event separating the seed-bearing pulp entirely from the pericarp. The hollow condition is especially a feature of tomato varieties yielding bilocular fruits, as the fruit wall is less rigidly supported by the carpels than is the case with the fruit of tri- or multi-locular varieties. As a consequence of this, when hollowness is associated

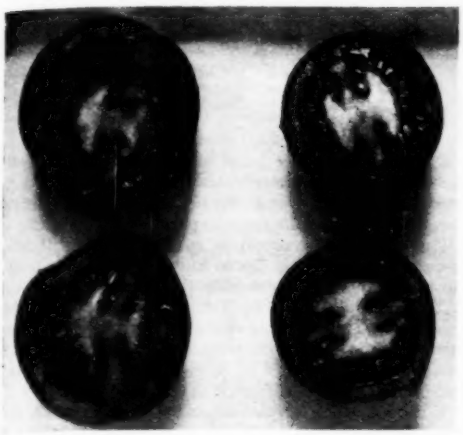

Fig. 1. Left, hollow bilocular fruit; right, normal bilocular fruit

with bilocular fruits, they frequently assume a flattened, angular appearance. This misshapen appearance, coupled with a lack of firmness and poor eating qualities, makes for low market value.

While investigating several aspects of the hollow fruit problem the relationship between this and tomato mosaic virus infection was examined. From a series of glasshouses, sparsely infected with virus, pairs of infected and virus-free plants were contrasted. Ten such random pairs were examined in each of twelve widely separated glasshouses. In all cases the numbers of hollow tomatoes borne on four consecutive trusses were compared with the total number of tomatoes on the same four trusses.

The results are presented in Table 1.

Table 1

Series	Plants	No.	Ratio sound/hollow tomatoes	Standard deviation	Difference (per cent)
1	T.M.V. infected	10	6·45	± 0·96	66·2*
	Non-infected	10	19·35	± 3·95	
2	T.M.V. infected	10	6·97	± 1·50	37·4
	Non-infected	10	11·14	± 3·30	
3	T.M.V. infected	10	2·24	± 0·12	49·6*
	Non-infected	10	4·45	± 1·00	
4	T.M.V. infected	10	3·96	± 0·66	12·2
	Non-infected	10	4·51	± 0·34	
5	T.M.V. infected	10	2·99	± 0·54	51·5
	Non-infected	10	6·17	± 2·02	
6	T.M.V. infected	10	1·54	± 0·13	18·5†
	Non-infected	10	1·89	± 0·09	
7	T.M.V. infected	10	6·33	± 1·10	67·9†
	Non-infected	10	19·72	± 5·80	
8	T.M.V. infected	10	2·60	± 0·45	16·1
	Non-infected	10	3·10	± 0·18	
9	T.M.V. infected	10	5·03	± 0·72	37·9†
	Non-infected	10	8·11	± 1·08	
10	T.M.V. infected	10	4·67	± 1·20	53·5
	Non-infected	10	10·06	± 4·44	
11	T.M.V. infected	10	3·33	± 0·40	42·2†
	Non-infected	10	5·76	± 1·06	
12	T.M.V. infected	10	7·00	± 1·25	32·0
	Non-infected	10	10·30	± 4·93	

* Significant differences at $P = 0{\cdot}01$
† Significant differences at $P = 0{\cdot}05$

The standard deviations obtained from the data collected from non-infected plants are variable and frequently larger than those obtained from infected plants. It is likely that these high standard deviations indicate, in some cases, the fallibility of visual diagnosis of virus-infected plants. The more regular standard deviations occurring for infected plants, which are easily identified, support this theory.

Experimental methods utilizing inoculated plants would probably produce much more even data. Nevertheless, the results suggest that a relationship exists between tomato mosaic virus infection and the incidence of hollow fruit.

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Growth of Contagious Pustular Dermatitis Virus in Human Tissue Cultures

Contagious pustular dermatitis or 'orf' is a well-known disease of sheep which occasionally causes lesions, usually on the hands or face of people who work with sheep or their carcases. Such lesions may be difficult to distinguish from those caused by cowpox virus or by certain bacteria and fungi and a simple means of laboratory confirmation would be useful. Human infections may be confirmed by transmitting the virus to sheep and carrying out cross-immunity tests; but this is laborious and seldom used. A complement-fixation test using sheep material as antigen was used by Macdonald[1], but is not very sensitive. The virus will not grow in the developing chick embryo—which distinguishes it from cowpox virus—or in any of the smaller laboratory animals.

Plowright *et al.*[2] described the growth of contagious pustular dermatitis virus in sheep testis and kidney tissue culture. We have found that a stock strain of the virus grows well in lamb embryo kidney tissue culture in monolayers. Using a medium containing 10 per cent calf serum we have obtained average titres of 10^6 fifty per cent tissue culture infectious doses per 0·1 ml. of infected fluids on repeated passage at 36° C.

The identity of the virus was checked by rubbing tissue culture fluid into the shaved skin of a non-immune sheep and re-isolating the virus in tissue cultures from typical 'orf' lesions. Cross-immunity tests using three sheep were suggestive but not entirely satisfactory, because a reduced number of lesions developed in both challenged animals which had been vaccinated either with tissue-culture vaccine or the standard Burroughs Welcome vaccine. Cytopathic changes in lamb embryo kidney cultures included early rounding and swelling of the cells followed by clumping which superficially resembles the appearances seen with certain types of adenovirus grown in human embryo kidney cells. With heavy inocula of the virus, cytopathic effect was seen after 24 hr. and most of the tissue cells infected became detached from the walls of the tubes in three days. The virus appears to reach maximum titre in cells after three days and is then liberated into the medium.

Contagious pustular dermatitis virus which had been passed three times in lamb tissue was used to infect stationary cultures of human amnion, human embryonic kidney and human embryonic liver cells. In all types of cell the virus grew well, causing cytopathic changes similar, in unstained preparations, to those seen in lamb kidney cultures. The medium used for the growth of the virus in human amnion cells, which are widely used in virus diagnostic laboratories, contained 10 per cent calf serum.

Titrations of the virus in tissue cultures and direct isolation of the virus from experimentally infected sheep both suggest that human amnion cells may be the most suitable available method for isolating contagious pustular dermatitis virus from human lesions. In preliminary tissue culture experiments we have not been successful in demonstrating unequivocally the development of neutralizing antibody after infection in either sheep or humans. Using an antigen derived from infected lamb embryo kidney cultures we have shown that experimentally infected sheep develop significant rising titres, up to 1 : 64, of complement fixing antibody three weeks after infection. 'Orf' is locally common in certain parts of England and Scotland, but in our area it is rare and we have not yet had the opportunity to examine adequate specimens from the infection in man.

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AGRICULTURE

A New Method of Estimating the Dry-Matter Intake of Grazing Sheep from their Fæcal Output

In a series of investigations on dry-matter intake and fæces output of ewes, at different feed-levels, it was observed that a particular fraction of the fæces, readily dissolved by treatment with dilute acid, was closely related to the intake of dry matter. It was considered that this dissolved fæcal fraction would be unlikely to contain any high proportion of food residues already subjected to fermentation and digestion in the alimentary tract but was likely to consist largely of the more soluble parts of the metabolic fraction of the fæces. It has already been shown that this fraction bears a close relationship to dry matter intake[1,2].

Total collection of the fæces was employed[3] and 1 gm. of the milled dried fæces was allowed to stand in 150 ml. of 0·2 *N* hydrochloric acid (containing a small quantity of wetting agent) at room temperature for 18 hr. After filtering through a paper disk in a Gooch crucible the amount of this dissolved fæcal fraction was determined and the total daily production calculated.

Further work during 1959 and 1960 involved a range of feeds, including fresh and dried herbage, fed at different levels of intake to ewes and wethers. For eleven feeds, including grass–clover mixtures, each fed to several animals, the relationship between daily dry-matter intake and the daily dissolved fæcal fraction output in gm. for 64 observations was $y = 250 + 17{\cdot}8\,x$, y being the predicted dry-matter intake and x the dissolved fæcal fraction output.

In the cases of two other legume feeds, lucerne and a clover mixture, dissolved fæcal fraction outputs were higher than those for grass and grass–legume mixtures at comparable levels of dry-matter intake.

The errors associated with the prediction of other y values for two values of dissolved fæcal fraction output for means of various numbers of animals are shown in Table 1.

Table 1

No. of animals in the group for which the prediction is made	Dissolved fæcal fraction daily output (gm.) 50 *S.E.* of estimate	50 Percentage error	100 *S.E.* of estimate	100 Percentage error
1	73·5	6·4	78·1	3·9
5	33·9	3·0	43·0	2·1
10	25·0	2·2	36·4	1·8
20	18·9	1·7	32·5	1·6

These levels of precision suggest that this method should be suitable for estimating the free grazing intake of ewes, without the need to develop separate 'local' regressions for each type of sward grazed.

This method overcomes some of the difficulties associated with some 'fæcal index' regressions used in the past since it is based on the relationship of dry-matter intake and dissolved fæcal fraction output, thus avoiding the error involved in applying regressions of fæces composition on dry-matter digestibility, developed at one level of intake indoors, to studies of grazing animals at different levels of intake.

Where the basic relationship is between the total output of a fæces component and feed intake, and the regression does not pass through the origin, fæces composition at any one level of feed digestibility will vary with intake.

These investigations are being extended and this method applied, in observations on grazing ewes, with the aid of a grant from the Agricultural Research Council.

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Groundnut Variety, Mani pintar

Smartt, in a recent communication[1], mentions the very satisfactory performance of the groundnut variety, Mani pintar, in trials in Northern Rhodesia. In a series of trials on agricultural stations and on local farms in the Northern Region, Ghana, in 1960 the variety was outstanding, giving a mean yield 35 per cent higher than the local varieties, 24 per cent higher than previously released improved varieties and out-yielding all other recent introductions.

The variegated red and white testa characterizing the variety was found to be quite acceptable to the local farmer, who uses most of the crop for expression of the oil.

It is suggested that a variety with many excellent qualities and which has performed well in two widely separated African territories is worthy of widespread trial in other groundnut-growing areas.

I am grateful to Mr. Smartt for providing the original seed for my work.

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, October 9

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. B. Mayo: "The Dilemma of Determinism".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the EWELL COUNTY TECHNICAL COLLEGE and the KINGSTON TECHNICAL COLLEGE CHEMICAL SOCIETIES, at the Ewell County Technical College, Reigate Road, Ewell, Surrey), at 7 p.m.—Prof. G. E. Coates: "Organic Derivatives of Coinage Metals".

Tuesday, October 10

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. S. Elliott: "Trends in Computer Engineering" (Chairman's Address).

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—"The Importance of the Design Stage" (Chairman's Address).

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Lecture by Dr. H. M. Talen (Paint Research Institute, Delft).

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the BRIGHTON TECHNICAL COLLEGE CHEMICAL SOCIETY, at Brighton Technical College, Brighton), at 7 p.m.—Prof. R. L. Wain, F.R.S.: "Chemical Control of Plant Growth".

Wednesday, October 11

SCHOOL OF PHARMACY, University of London (at 29–39 Brunswick Square, London, W.C.1), at 3 p.m.—Sir Eric Ashby: "Placebo and Analgesic in Education".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. Arne Noë-Nygaard (University of Copenhagen): "Geology of the Faeroes" (Sixteenth William Smith Lecture).

BRITISH COAL UTILIZATION RESEARCH ASSOCIATION (in the Conway Hall, Red Lion Square, Holborn, London, W.C.1), at 5.30 p.m.—Mr. L. Rotherham: "The Position of Coal in Electric Power Generation" (Tenth Coal Science Lecture).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Scientific Papers.

INSTITUTE OF METAL FINISHING, ORGANIC FINISHING GROUP (at the British Institute of Management, 80 Fetter Lane, London, E.C.4), at 6.30 p.m.—Mr. D. H. Grover: "Plastic Industrial Paints".

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. H. B. Whitehouse and Mr. M. I. MacLaurin: "Process Control in Paper Mills".

Thursday, October 12

INSTITUTE OF REFRIGERATION (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. C. M. Brain: Presidential Address.

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. H. G. Taylor: "Research in the Field of Electricity Utilization" (Chairman's Address).

RADAR AND ELECTRONICS ASSOCIATION (in the Lecture Hall of the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p.m.—Mr. L. F. Matthews: Space Communications. Part 1: Systems and Equipment".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Sir George McRobert: "From Empire to Commonwealth" (Presidential Address).

Friday, October 13

ASSOCIATION OF APPLIED BIOLOGISTS (at the British Museum (Natural History), South Kensington, London, S.W.7), at 10.50 a.m.—Meeting on "Mites of Economic Importance".

Saturday, October 14

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. J. A. Fraser Roberts: "The Usefulness of the Blood Groups and some other Simply Inherited Characteristics in Anthropological Studies".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (Grade B) IN MATHEMATICS—The Registrar, Flintshire Technical College, Connah's Quay, near Chester (October 13).

ASSISTANT LECTURER IN ZOOLOGY—The Registrar, The University, Nottingham (October 14).

ASSISTANT LIBRARIAN (Acquisitions) (honours graduate with professional qualifications in librarianship and practical experience in an academic library)—The Secretary of University Court, The University, Glasgow, W.2 (October 14).

LECTURER or ASSISTANT LECTURER (with an honours degree and practical experience of at least one branch of the food industry) IN FOOD SCIENCE in the Proctor Department of Food and Leather Science—The Registrar, The University, Leeds 2 (October 14).

TUTORIAL STUDENT (graduate or about to graduate) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (October 14).

POSTDOCTORAL RESEARCH ASSISTANT, to work on high energy physics using the nuclear emulsion technique—Prof. E. H Bellamy, Department of Physics, Westfield College (University of London), London, N.W.3 (October 20).

SCIENTIFIC ASSISTANT IN THE CHEMISTRY DEPARTMENT, for the maintenance of physical instruments including spectrophotometers and radiochemistry equipment; duties will also include certain routine measurements and demonstrations—Head of the Department of Chemistry, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (October 21).

ASSISTANT LECTURER or LECTURER (preferably with a special interest in analysis or algebra) IN PURE MATHEMATICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (October 23).

CHEMIST, CHEMICAL ENGINEER, or PHYSICIST (with a university honours degree or equivalent in chemistry, chemical engineering or physics together with some years of research experience) IN THE CHEMICAL ENGINEERING SECTION, C.S.I.R.O., Melbourne, Australia, to undertake work of a fundamental nature in the fields of fluid dynamics, mass transfer and reaction kinetics—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 608/15 (October 28).

RESEARCH FELLOW or SENIOR RESEARCH FELLOW IN THE DEPARTMENT OF PARASITOLOGY, University of Queensland, for duties which will include the instigation of research work on pathogenesis of parasitic infections in domestic animals, and also a certain amount of undergraduate teaching—The Registrar, University of Queensland, Brisbane, Queensland, Australia (October 28).

ASSISTANT SYSTEMATIC BOTANIST (honours graduate in botany or agricultural botany)—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (October 30).

LABORATORY SUPERINTENDENT IN THE DEPARTMENT OF ENGINEERING, Fourah Bay College, University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 30).

CHAIR OF CHEMICAL PATHOLOGY—The Secretary, The University, Aberdeen (October 31).

CHEMIST (well qualified, with an interest in microbiology and enzyme chemistry), for research relating to the production of slipe wool—The Secretary, Wool Research Organization of New Zealand (Inc.), P.O. Box 5115, Wellington, New Zealand, or the New Zealand Scientific Liaison Officer, Africa House, Kingsway, London, W.C.2 (October 31).

DEPUTY (preferably with a degree in zoology) TO THE DIRECTOR, Whipsnade Park, to assist the Director in the scientific and animal management work in the Park—The Secretary, Zoological Society of London, Regent's Park, London, N.W.1 (October 31).

LECTURER or ASSISTANT LECTURER IN ANATOMY at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 31).

LECTURERS (2) (with special qualifications and experience in the food industry (other than the dairy industry) or in biochemical engineering) IN FOOD SCIENCE—The Registrar (Room 22, O.R.B.), The University, Reading (October 31).

PROFESSOR OF MATHEMATICS or PROFESSOR OF APPLIED MATHEMATICS at the University of Natal, Durban—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, October 31).

SENIOR LECTURER and a LECTURER IN MATHEMATICS at the University College of Townsville, University of Queensland—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 31).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 5).

CHAIR OF PHYSICS in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 10).

LECTURER IN PSYCHOLOGY in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 15).

LECTURER IN PHILOSOPHY at Newcastle University College, The University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and the Appointments Section, Box 1, Post Office, Kensington, New South Wales, Australia (November 17).

LECTURER IN ANATOMY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 18).

LECTURER/SENIOR LECTURER (graduate with an appropriate degree and with postgraduate research and/or field experience in agronomy) IN AGRONOMY at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

ASSISTANT PROFESSOR IN PHARMACOLOGY—Prof. M. F. Murnaghan, University of Ottawa, Ottawa, Ontario, Canada.

EXPERIMENTAL OFFICER (with a pass degree including chemistry or H.N.C. in chemistry) in County Hall Laboratory, for duties which will involve research, control and development in a wide field of science—The Medical Officer of Health (D.1/N/2598/9), County Hall, London, S.E.1.

HEAD (with good qualifications in chemistry and teaching experience) OF THE SCIENCE DEPARTMENT—The Headmistress, Roedean School, Brighton.

KEEPER (with an honours degree and/or a museums diploma) OF ETHNOLOGY—The Director, Liverpool Museums, William Brown Street, Liverpool 3.

LABORATORY TECHNICIAN, for a research laboratory specializing in lipid analysis for metabolic studies—J. A. Balint, M.B., M.R.C.P., Division of Gastroenterology, University of Alabama Medical Center, Birmingham 3, Alabama, U.S.A.

LECTURER or SENIOR LECTURER IN EDUCATION—The Principal, The James Graham College, Lawns House, Chapel Lane, Farnley, Leeds 12.

LECTURER (preferably with experience in circuit theory and able to supervise research) IN THE FIELD OF LIGHT ELECTRICAL ENGINEERING—Head of the Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7.

LECTURER (with an honours degree in chemical engineering or Corporate Membership of the Institution of Chemical Engineers, with experience in research and in the operation and supervision of full-scale chemical plant) IN CHEMICAL ENGINEERING—The Registrar, The University, Nottingham.

MASTER TO TEACH CHEMISTRY to all levels, including a substantial amount of sixth form work—The Headmaster, King's College School, Wimbledon, London, S.W.19.

MATHEMATICIANS (with an honours degree in mathematics, and preferably previous experience with computers) IN THE COMPUTATION CENTRE, National Research Council of Canada, for work which will involve the analysis and programming of problems which arise in the various scientific and engineering divisions of the Council—The Employment Officer, National Research Council, 100 Sussex Drive, Ottawa, Ontario, quoting Ref. E.R.210.

RESEARCH STUDENT IN INORGANIC CHEMISTRY, for work concerned with inorganic polymers—The Professor of Chemistry, Westfield College (University of London), Hampstead, London N.W.3.

RESEARCH STUDENTS IN THE DEPARTMENT OF CHEMISTRY, for work on the inorganic chemistry of complexes in solution, polyacids, etc.—The Registrar, Manchester College of Science and Technology, Sackville Street, Manchester 1.

TECHNICAL ASSISTANT (with a university degree or equivalent qualifications, and the ability to assist in the construction of research apparatus) IN THE DEPARTMENT OF APPLIED PHYSICS, to assist in research on subjective brightness—Head of the Applied Physics Department, Northampton College of Advanced Technology, St. John Street, Clerkenwell, London, E.C.1.

REPORTS and other PUBLICATIONS

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Privy Council. Medical Research Council Memorandum No. 40 : Ageing and the Semi-skilled—a Survey in Manufacturing Industry on Merseyside. By Alastair Heron and Sheila M. Chown, assisted by M. S. Featherstone and Catherine M. Cunningham. Pp. viii + 59. (London : H.M. Stationery Office, 1961.) 5*s*. net. [148

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Department of Scientific and Industrial Research : Road Research Laboratory. Road Research Technical Paper No. 51 : Factors Affecting the Amount of Travel. By J. C. Tanner. Pp. iv + 26. (London : H.M. Stationery Office, 1961.) 2*s*. net. [148

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